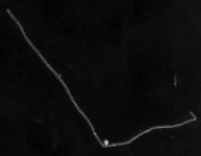


JOURNAL
OF THE
FISHERIES RESEARCH BOARD
OF CANADA

Benthic Infauna Communities off the Coast of Washington and in Puget Sound: Identification and Distribution of the Communities

ULF LIE AND JAMES C. KELLEY

Department of Oceanography
University of Washington, Seattle, Wash. 98105, USA

LIE, U., AND J. C. KELLEY. 1970. Benthic infauna communities off the coast of Washington and in Puget Sound: identification and distribution of the communities. J. Fish. Res. Bd. Canada 27: 621–651.

Benthic infauna was collected at 37 stations in Puget Sound, in Juan de Fuca Strait, and off the northwestern coast of Washington during the summer of 1967, and at 18 additional stations off the southwestern coast of Washington during the summer of 1968. The crustaceans, lamellibranchs, and echinoderms were identified and counted, and the data were subjected to an analysis of affinity among stations, to Fager's recurrent group analysis, and to factor analysis for identification of the benthic communities and the groupings of benthic species in the investigated area.

Trellis-diagrams of indices of affinity among the stations, based on the presence or absence of species, indicated four rather distinct groups of stations with specific geographic distributions or relations to certain sediment types. Fager's recurrent group analysis for the 36 most frequently occurring species resulted in eight groups and five species that were strongly associated with one or more of the recurrent groups. Most tests of interspecific relations among the species within the groups were not significant, and it was concluded that the analysis was only partly useful for delimiting species groups with ecological significance. The Q-mode of the factor analysis resulted in three groups of stations distributed in bands parallel to the coast, and each group of stations (community) could be recognized by its distinct sediment types or depth distribution. The R-mode of the factor analysis gave six groups of species with specific preferences of depths, sediment types, or geographic distribution. The results of the various techniques were compared, and their application in benthic synecology was discussed.

Received October 29, 1969

INTRODUCTION

DURING THE FIRST HALF of the 20th century, a number of investigators around the world attempted to map the benthic communities according to the methods developed by Petersen (1913, 1918). The communities were regarded as convenient statistical units for mapping purposes, or as fundamental ecological units, biocoenoses, with well-defined internal regulation of composition (Thorson, 1957). There was considerable discussion about whether the communities were determined primarily by hydrographic parameters (Molander, 1928) or by chemical and physical properties of the bottom sediments (Jones, 1950); but as there was no quantitative measurement of community composition, one could not quantitatively measure relations among communities, nor estimate the relative influence of environmental parameters on the composition and distribution of the communities.

Printed in Canada (J1635)

The occurrence of discrete, well-defined benthic communities was questioned because of the apparent lack of distinct boundaries between them (Lindroth, 1935). In terrestrial plant ecology the concept of "continuum" was developed, i.e., the qualitative and quantitative composition of biotic assemblages (communities) is changing continually along environmental gradients (Gleason, 1926). Naturally, the concept of "continuum" is poorly suited for classification purposes, but it stimulated the search for mathematical methods to measure synecological relationships, and this eventually led to ordination of the flora in multidimensional coordinate systems with environmental gradients as axes (Whittaker, 1967). The mathematical methods are of two types; one based only on presence or absence of species, the other also taking into account the abundance of the various species. Furthermore, the methods measure similarities in species composition among stations, or distributional similarities among species.

A method for measuring affinity between faunal assemblages, based on Kendall's rank correlation coefficient (Kendall, 1962), was used in a study of prairie vegetation by Looman and Campbell (1960). Lie (1968) used this method for a study of the similarity in composition of benthic infauna among eight permanent stations in Puget Sound during 1963–64, and found that the stations could be ordinated along a gradient of the particle-size distribution of the sediments. Nichols (1970) applied the technique to polychaete assemblages from nine closely spaced stations in Puget Sound and found that the arrangement of the stations based on the index of affinity was strongly correlated to depths and clay percentage of the sediments.

Fager (1957) suggested a method, the recurrent group analysis, for grouping of organisms based on the presence or absence of species at the stations. The method was slightly improved, programmed for the CDC 1604 computer, and used in a study of zooplankton from the Pacific Ocean by Fager and McGowan (1963). The recurrent group analysis yields groups of species that frequently occur together; and a number of tests based on rank correlations were suggested for studies of relationships, such as dominance and distributional similarities among the species within the groups. The method has been used successfully for grouping of zooplankton (Fager and McGowan, 1963; Sheard, 1965), demersal fishes (Fager and Longhurst, 1968), and for invertebrates in decaying wood (Fager, 1968).

Various branches of multivariate analysis (Kendall, 1957; Anderson, 1958) have recently been used in quantitative synecology; these methods also consider the abundance of the various species. The multivariate analyses in ecology yield results of two kinds (Greig-Smith, 1964): the R-mode for ordination of species in a multidimensional space determined by the stations, and the Q-mode for ordination of stations in a space determined by the species. Multivariate analyses were first used for identification of terrestrial plant communities; Goodall (1954) used principal component analysis, and Dagnelie (1960, 1965) used factor analysis and canonical correlation for ordination of vegetation in relation to environmental parameters. In marine biology, principal

component analysis has been used by Williamson (1961) for a study of the relation between zooplankton and hydrography, by Williamson (1963) for a study of the relations between herring and zooplankton, and by Colebrook (1964) for a study of the geographical distribution of zooplankton in British waters. Cassie and Michael (1968) used principal component analysis and canonical correlation for intertidal benthic fauna from New Zealand and found that the resulting communities were strongly correlated with sediment parameters and compared favorably with the results of classical community analyses of the same data.

The present paper reports on the use of various mathematical techniques for determining the distribution and composition of benthic communities in Puget Sound and off the coast of Washington. The study is intended as an introduction to future long-term investigations of the productivity of benthos in the area. The benthic fauna in this area is often characterized by lack of large, conspicuous species with significant contribution to the total biomass; and therefore classical community analyses based on characteristic species (Thorson, 1957) cannot be performed. Furthermore, classical analyses result in discrete communities, whereas in modern synecology communities are conceived of as stages in a "continuum" (Whittaker, 1967). Therefore, ordination in a space determined by environmental gradients may provide a better understanding of the synecological relations than classification techniques. In the present paper the mathematical analyses are attempts partly to classify the benthic communities and partly to ordinate the communities in a multidimensional space with the environmental parameters as axes.

MATERIAL AND METHODS

Sampling, Sorting, Identification, and Enumeration of the Fauna

Benthos samples were collected with a 0.2-m^2 van Veen grab weighing 50 kg at 37 stations in Puget Sound, Juan de Fuca Strait, and off the northwestern coast of Washington during May–August 1967, and at 18 stations off the southwestern coast of Washington during July 1968 (Fig. 1). The ship was anchored during sampling. At 48 stations three samples were collected, at 5 stations two samples were collected, and at 2 stations only one sample was collected. A subsample for particle-size analysis of the sediments was taken from each grab sample. The grab contents were sieved through a 1-mm stainless steel screen, and the remains on the screen were preserved in 4–10% formalin buffered with sodium borate, $Na_2B_4O_7$. In the laboratory the samples were sorted into Polychaeta, Crustacea, Mollusca, Echinodermata, and "Miscellaneous," and the weights of the various groups were determined (Lie, 1969). All the crustaceans, lamellibranchs, and echinoderms (127 species) were identified and counted, and the data were subjected to the various mathematical analyses as discussed below. References for the identification of the species can be found in Lie (1968).

Polychaeta, which contributed significantly to the standing crop in the area (Lie, 1969) were not identified; however, the dominant polychaete species of the various communities will be included in a paper about the structure of the communities that will be published later.

Particle-Size Analyses of the Sediments

The sediment samples were homogenized and subjected to routine particle-size analyses as described by Krumbein and Pettijohn (1938). The gravel and sand fractions (particles ≥ 0.063 mm) were divided into six size classes by dry sieving through a series of screens, and the silt and

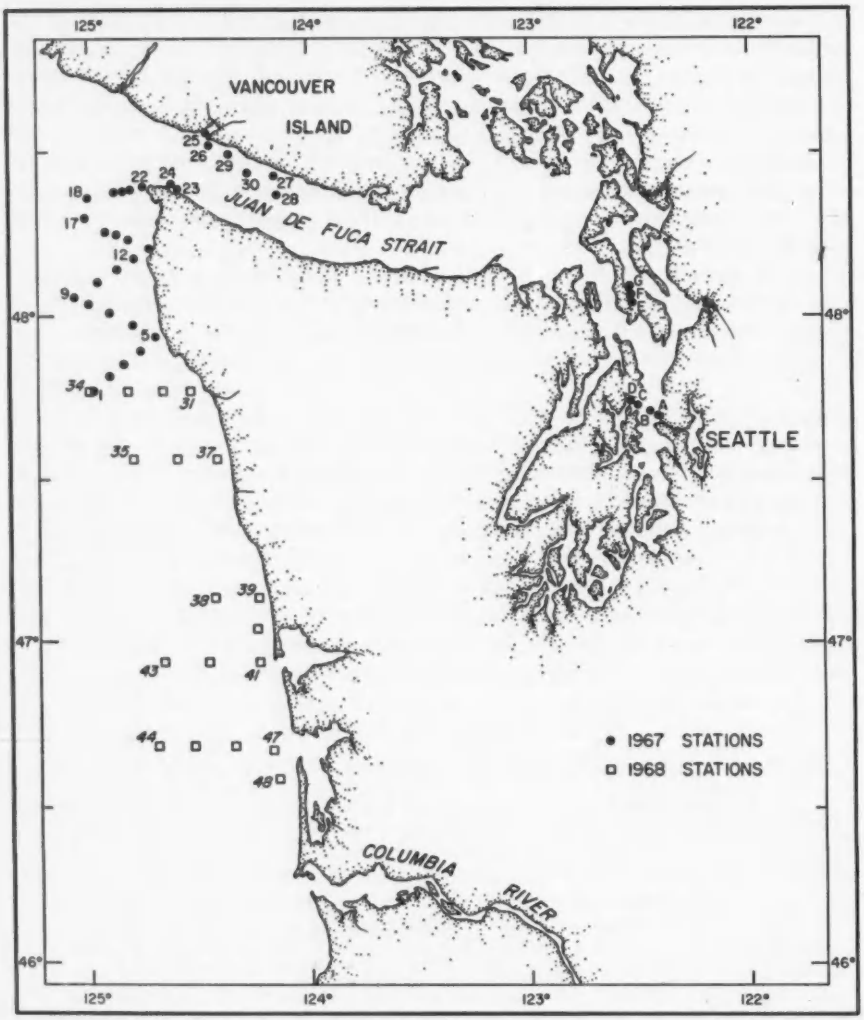


FIG. 1. Distribution of the benthos stations.

clay fractions were divided into eight size classes by pipetting. The weights of the various size classes were subjected to statistical analyses for estimating various parameters of the particle-size distributions by a computer program by Creager et al. (1962).

THE INDEX OF AFFINITY

The index of affinity is Kendall's rank correlation coefficient for maximally tied ranks (Kendall, 1962). If in a set of stations there are S species, and at stations i and j there are A_i and A_j species, respectively, and stations i and j have C_{ij} species in common, the data may be expressed in a 2 × 2 contingency table as:

C_{ij}	V_i	A_i
V_j	W_{ij}	X_i
A_j	X_j	S

where X_i and X_j are the number of species not occurring at stations i and j; V_i (V_j) are the number of species found at station i (j) but not at station j (i); W_{ij} is the number of species out of S that do not occur on either i nor j. The index of affinity between stations i and j is then:

$$T_o = (C_{ij}W_{ij} - V_iV_j)/(X_iA_i \cdot X_jA_j)^{1/2}.$$

The square contingency of the 2 × 2 contingency table is

$$\text{chi}^2 = (C_{ij}W_{ij} - V_iV_j)^2S/(X_iA_i \cdot X_jA_j)$$

or:

$$T_o = (\text{chi}^2/S)^{1/2}.$$

If chi^2 for a certain level of probability (say $P = 0.05$) with one degree of freedom is entered in the equation, then $T_e = (\text{chi}^2_{0.05}/S)^{1/2}$ is the smallest value of the index of affinity that indicates significant affinity on the 95% level of probability, and all values of $T_o \geq T_e$ indicate significant affinity between pairs of stations.

Calculations of T_o were programmed for the IBM 7094 computer and estimated for all possible pairs of the 37 stations, based on all the 127 species. The resulting matrix of affinity coefficients was arranged to show groupings with strong affinities according to standard methods for arranging trellis-diagrams (Macfadyen, 1963), but these methods become extremely cumbersome when the number of stations is high, and there is a certain element of subjectivity involved. An arrangement that is objective and is an approximation to the standard methods for arranging trellis-diagrams has therefore been programmed for the IBM 7094 computer. The station with the lowest sum of affinities to the other stations is placed first in the matrix, and the station with the highest affinity to the first station is placed second in the matrix. The station with the highest affinity to the second station is placed third, unless there is another station that has an affinity to the first station ≥ 1.5 times the affinity of the third station to the first station. If so, that station is entered as the third station. The fourth station to be entered is the one with the highest affinity to the third station, unless there is another station that has an affinity to the second station 1.5 times higher than the affinity of the fourth station to the second station, etc.

Fager's Recurrent Group Analysis

Fager (1957) suggested a method for grouping of species based on a measure of similarities in distribution among species. The measure of similarity between two species A and B was modified by Fager and McGowan (1963) to:

$$I = J/(N_AN_B)^{1/2} - 1/2(N_B)^{1/2}$$

N_A and N_B are the number of stations where species A and B occurred, respectively, and J is the number of stations where the two species occurred together. It was assumed that two species should have joint occurrences on at least half of the stations in order to be considered associated, and therefore a value of $I \geq 0.5$ indicated significant affinity. Interspecific relations in the resulting recurrent groups were tested by various rank-correlation tests (Fager, 1957). Tests of concordance among rankings of species abundances (Kendall, 1962) were used to determine whether there was agreement among the species in a group as to what constituted a "good"

environment, and to determine dominance relations among the species. Kendall's rank correlation coefficient, τ, was suggested as a test of distributional similarity between pairs of species in a recurrent group. For specifics about the estimation of recurrent groups and the corresponding tests, see Fager (1957).

The recurrent group analysis was first applied to the 95 species in the present material that occurred at two or more stations, and with $I = 0.5$ as limiting value for significant affinity. The result was 4 groups of 3 species, 13 groups of 2 species, and 57 species without significant affinity with any other species. It is possible that $I = 0.5$ was too restrictive when the number of stations was small (only two species occurred on more than 50% of the stations), and therefore many of the species occurred on too few stations to yield significant information. For this reason, the program was rerun with $I = 0.333$ indicating significant affinity, using only the 36 species that occurred on more than 15% of the stations (Table 1). The same species were subjected to factor analyses.

TABLE 1. Mean abundance ($\overline{X}$) and frequency of occurrence (F) of the most common species in the 1967 data.

Species	$\overline{X}$	F	Species	$\overline{X}$	F
Ampelisca macrocephala Lilljeborg	22.2	29	*Paraphoxus obtusidens* (Alderman)	5.2	10
Axinopsida sericata Carpenter	10.3	24	*Nucula bellotii* Adams	0.9	10
Ampelisca compressa Holmes	3.9	16	*Monoculodes spinipes* Mills	1.5	8
Heterophoxus oculatus (Holmes)	4.0	15	*Acila castrensis* (Hinds)	2.8	7
Photis brevipes Shoemaker	3.8	15	*Adontorhina cyclia* Berry	1.4	8
Amphiodia urtica (Lyman)	2.7	14	*Compsomyax subdiaphana* (Carpenter)	0.4	8
Macoma elimata Dunnill & Ellis	1.0	13	*Tellina buttoni* Dall	6.6	8
Macoma carlottensis (Whiteaves)	15.8	13	*Amphipholis squamata* (Delle Chiaje)	2.8	8
Byblis veleronis Barnard	3.3	11	*Euphilomedes producta* Poulsen	4.5	7
Paraphoxus variatus Barnard	6.1	13	*Westwoodilla caecula* (Bate)	2.3	7
Pinnixa schmitti Rathbun	5.0	12	*Diastylis alaskensis* Calman	1.3	8
Diastylopsis dawsoni Smith	243.4	16	*Nuculana minuta* (Fabricius)	1.9	7
Tellina carpenteri Dall	0.8	11	*Yoldia ensifera* Dall	10.7	9
Mysella tumida (Carpenter)	1.8	10	*Nemocardium centifilosum* (Carpenter)	0.4	6
Psephidia lordi (Baird)	6.6	10	*Pandora filosa* (Carpenter)	0.5	7
Amphioplus sp.	1.0	10	*Melita dentata* Krøyer	1.0	6
Ophiura lütkeni (Lyman)	1.7	10	*Diastylis pellucida* Hart	0.4	6
Paraphoxus abronius Barnard	3.0	8	*Eudorella pacifica* Hart	0.3	6

The computer program for Fager's recurrent group analysis results in grouping of the species (REGROUP), a measure of affinities among the groups (CONNEX), and a list of stations at which the various recurrent groups occur (STATION).

FACTOR ANALYSIS

The matrices of correlation coefficients among species and among stations were analyzed by factor analysis in the R- and Q-modes. In the R-mode analysis, the correlation coefficients among species were computed as

$$r_{ij} = \frac{\sum_{k=1}^{n} (x_{ik} - \bar{x}_i)(x_{jk} - \bar{x}_j)}{(n-1)s_i s_j} \qquad \begin{matrix} i,j = 1,2,\ldots m \\ i \neq j \end{matrix}$$

where m is the number of species, n is the number of stations, x_{ik} is the abundance of species i at station k, $\bar{x}_i$ is the mean abundance of the ith species, and s_i is the standard deviation of the x_{ik} measured over the n stations. The matrix $R_{m \times m} = \{r_{ij}\}$ was computed, and ones were placed on the principal diagonal (Cattell, 1965a, b). The values for species counts were transformed by the equation

$$x_{ik}^{*} = \ln (x_{ik} + 1)$$

where x^{*} is the transformed value of x, in order to give variances for the species that were independent of the means (Bartlett, 1947) in the R-mode analysis. The matrix was then analyzed for principal components by determining its positive eigenvalues and the associated eigenvectors. The first five principal components were selected for rotation and interpretation. Rotation was done using the varimax criterion (Kaiser, 1958). The five rotated factors were used for ecological interpretation.

In the Q-mode the data were transformed by the percent range transformation

$$x_{ik}^{*} = \left[(x_{ik} - x_{i\min}) / (x_{i\max} - x_{i\min}) \right] \times 100$$

where $x_{i\min}$ and $x_{i\max}$ are the minimum and maximum values of the ith species counted on the set of stations (Imbrie and van Andel, 1964). This transformation has the effect of treating very abundant species (in terms of counts) equally with very sparse species.

In the Q-mode the factor matrix is computed as

$$Q = \{\cos \theta_{kk'}\}$$

where $\cos \theta_{kk'}$ is the cosine of the angle between the vectors representing stations k and k' in the variable space (Imbrie and van Andel, 1964).

In the Q-mode the analysis was carried out as in the R-mode, but only three principal components were retained for rotation.

The eigenvalues were determined (according to the Jacobi diagonalization method (Harman, 1960)) by successive orthogonal transformations of the form

$$D_k = B'_{ij} \, RB_{ij},$$

which transforms the ij element of R to zero. The method converges to a solution of the form

$$D = B'RB$$

where

$$D = \text{diag} \, (\lambda_1, \lambda_2, \ldots, \lambda_n)$$

where the λ_i are the eigenvalues of R and

$$B = [\xi_1, \xi_2, \ldots, \xi_n]$$

where the ξ^i are column eigenvectors of R; B′ is the transpose of B. The method is derived from the relation

$$R\xi_i = \lambda_i \xi_i \qquad i = 1, 2, \ldots, n,$$

which is the definition of the eigenvector ξ_i. Writing the eigenvector matrix B we have

$$RB = BD$$

or

$$B^{-1}RB = D.$$

Since B represents a series of orthogonal transformations,

$$B' = B^{-1}$$

and

$$B'RB = D$$

the matrix of factor loadings is computed as

$$A = B'D^{1/2}.$$

The eigenvectors span the n-space of the stations, but a smaller basis that also effectively spans it may often be found. A likely set of basis vectors is the first k columns of B, where k is chosen by examining the relative successive decrease in the λ_i. In this study, k was set at 5 and thus the matrix of factor loadings, A, was reduced from $m \times m$ to $m \times 5$ by eliminating the last $m - 5$ columns.

The principal components are extracted because they can be determined uniquely and relatively easily. They express the orthogonal "qualities" common to the species or stations. They may not, however, be amenable to interpretation in a subject matter context, and rotation to a new and biologically more meaningful basis for the space may be carried out. In this study the rotation was made to satisfy the varimax criterion for "simple structure" (Kaiser, 1958). The criterion requires that the variance of the squared factor loadings of each be maximized. That is, for factor p

$$\sigma_p^2 = \frac{1}{m}\Sigma_j\left(b_{jp}^2\right)^2 - \frac{1}{m^2}\Sigma_j\left(\Sigma b_{jp}^2\right)^2 \quad p = 1,2,\ldots k$$

where the b_{jp} are the rotated factor loadings in the R-mode. Defining

$$\sigma^2 = \Sigma_p\sigma_p^2$$

the "raw" varimax criterion is

$$\sigma^2 \rightarrow \max.$$

Kaiser (1958) suggests a slight adjustment of the criterion to yield the "normal" varimax criterion,

$$V = n\left[\Sigma_p\Sigma_j\left(\frac{b_{jp}}{h_j}\right)^4 - \Sigma_p\left(\frac{\Sigma_j b_{jp}^2}{h_j^2}\right)^2\right]$$

where the h_j are the communalities measured over the k factors. This latter criterion was used to rotate the factors in the R-mode and an analogous equation for the Q-mode.

In Fig. 12 the vectors representing the stations in the three factor space are shown projected onto the unit sphere. The first octant of the unit sphere is shown as the triangle in the figure. This projection introduces some distortion, increasing with distance from the center of the triangle, but it circumvents the difficulties of examining the two-factor plots. The projection is gnomonic and centered at the point (1/3, 1/3, 1/3) in the ternary diagram.

The matrix $B = \{b_{jp}\}$ of the rotated factor loadings was used for interpretation of the analysis.

RESULTS

The Index of Affinity, T_o

The matrix of the index of affinity between all possible pairs of the 37 stations from 1967 was arranged by hand in a trellis-diagram according to

standard methods as described in Macfadyen (1963). This involved several trials and a certain degree of subjectivity, but the final arrangement (Fig. 2A) indicates four more-or-less distinct groups of stations and some stations that did not show strong affinity to more than one station. The first group (stations F–C) included all the stations from Puget Sound except station B; the second group (stations 10–9) included station B and the seven outermost stations

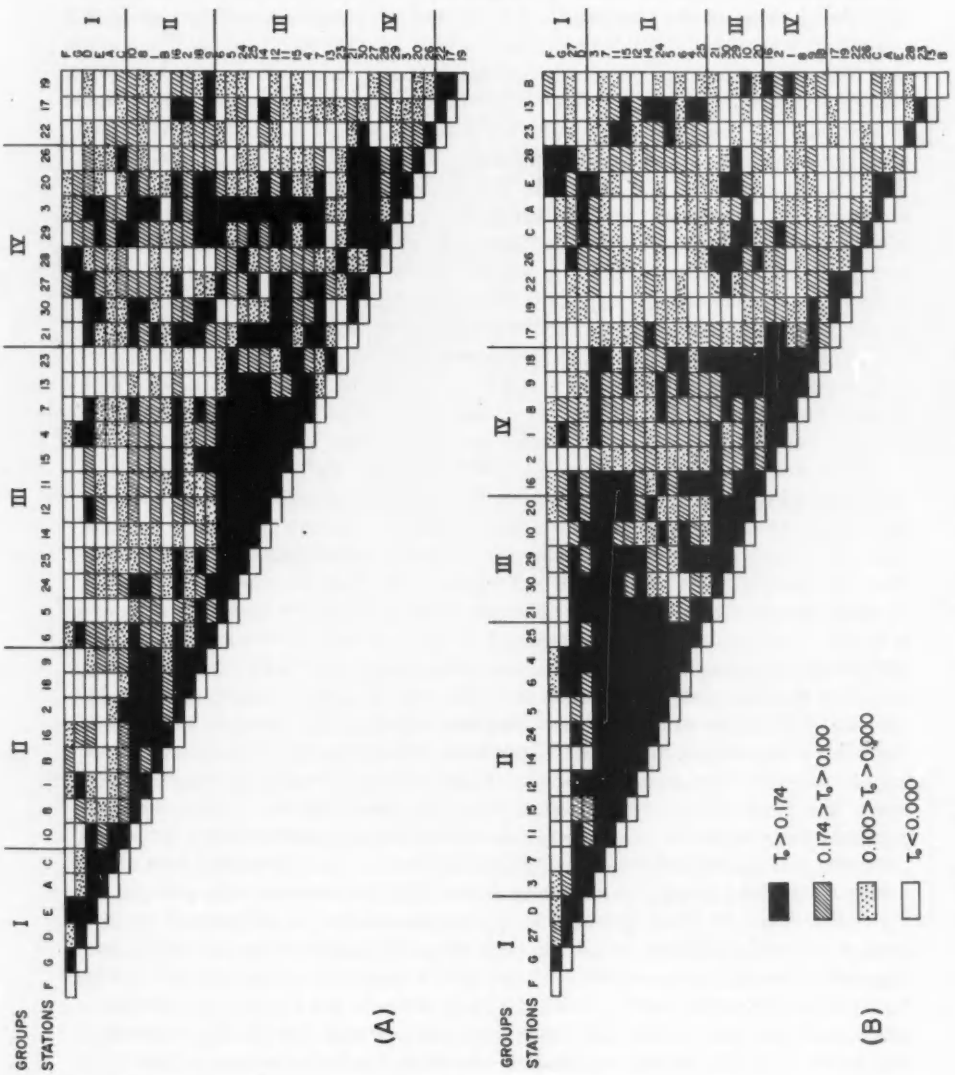


FIG. 2. Trellis-diagrams of indices of affinity arranged by hand (A) and by computer (B).

off the coast of Washington; the third group (stations 6–23) included eight stations closest to the shore off the coast and two shallow-water stations in the outer part of Juan de Fuca Strait; the fourth group (stations 21–26) included mainly stations from Juan de Fuca Strait. Three of the stations (stations 22, 17, 19) could not be assigned with certainty to any of the groups. The stations of the second group are characterized by sediments dominated by silt and clay, the stations of the third group have very high sand percentages, and the stations of the fourth group have relatively high percentages of both sand and mud.

The arrangement of the trellis-diagram by computer gave slightly different results (Fig. 2B), and the grouping of the stations was less distinct. The location of station 27 in the first group is highly questionable; it has, in fact, stronger affinities with the fourth group (Fig. 2A). The groups I, II, III, and IV in Fig. 2B are similar to the groups I, III, IV, and II in Fig. 2A, respectively. However, the groups in Fig. 2B include fewer stations, and a general feature of the matrix arranged by the computer is the grouping of all the stations with significant affinity only to one or a small number of stations in the right-hand corner. This group includes stations 17, 19, and 22, which were also left out of the groupings in Fig. 2A; and the latter figure shows that stations B and 28 could be assigned only with difficulty to a specific group. Figure 2A shows that station 13 and perhaps 23 could be assigned to the second group, and station 26 to the third group, whereas the three Puget Sound stations (A, C, and D) could not be assigned easily to any specific group.

The trellis-diagram arranged by hand (Fig. 2A) and the one arranged by computer (Fig. 2B) thus show largely the same groupings of stations, but the computer fails to assign the stations with low degrees of affinity to their appropriate groups. This is because the computer considers only affinity with the two nearest neighbors to the left in the data matrix, whereas arranging by hand permits many possible relations to be considered. It seems that arranging by computer is a very useful and time-saving preliminary step, and the resulting trellis-diagram can be rearranged easily by hand by considering relations as discussed above. Alternatively, the computer program could be modified to consider other relations, but the criteria rapidly become subjective. Certainly, rearranging by hand reintroduces the element of subjectivity but only to a small degree, since the major groups will be determined by the computer. The final arrangement could probably be done best by a person without any knowledge of the fauna or the environment at the various stations. Although the groups in most instances are easily identified as black triangles, it is often a major problem to decide where to draw the line between two groups. In Fig. 2A station 10 has high affinity to all the stations of the second group, station 6 has high affinity to all the stations in the third group, station 21 has high affinity to all but one station in the fourth group but only to seven of the 12 stations in the third group, and the lines between the groups are therefore rather well defined. In Fig. 2B the groups are less well defined, but station 3 has high affinity to all the stations in the second group, station 21 has high

affinity to all the stations in the third group, and station 16 has high affinity to all the stations in the fourth group.

Recurrent Group Analysis

The recurrent group analysis (REGROUP) of the 36 most frequently occurring species in the present material resulted in one group of six species, two groups of four species, three groups of three species, and two groups of two species. The analysis provides a measure of the affinity between groups (CONNEX), and the groups may be arranged in a diagram illustrating the affinity relations between the groups (Fig. 3). In the diagram only affinities ≥ 0.333

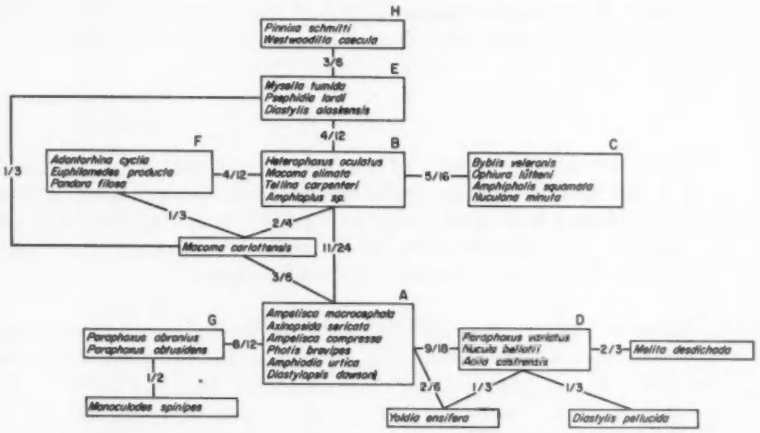


FIG. 3. Recurrent groups (REGROUP) and intergroup affinities (CONNEX) in the 1967 data.

have been considered. Eight of the 36 species were not included in any of the recurrent groups, but four of them had significant affinities to one or more members of the recurrent groups and have therefore been included in Fig. 3.

Fager and Longhurst (1968) were able to form assemblages with high affinities among recurrent groups within the assemblages and very low or no affinity among groups between the assemblages of demersal fishes from West Africa. Thus the assemblages indicated discrete communities correlated to depth zonation. Figure 3 shows that there is no basis for splitting the recurrent groups from the present material into distinct communities; rather the intergroup affinities leave the impression of a "continuum"; i.e., the recurrent groups are strongly interconnected. Four of seven recurrent groups of zooplankton from the Pacific Ocean (Fager and McGowan, 1963) showed similarly strong intergroup affinities; these groups were all distributed in the central and equatorial water masses. However, two other recurrent groups showed very low intergroup affinities, and one of these groups was distributed in the

transitional water masses between subarctic and central water masses and the other in subarctic water masses. The lack of faunal continuity as measured by intergroup affinities may therefore be related to environmental discontinuity.

The interrelationships among the groups demonstrated in Fig. 3 might be explained by studying the stations on which the various groups occurred (STATION). However, all the species, particularly of the larger groups, were found on too few stations to give meaningful distributions, and therefore stations where a high proportion of the recurrent group was found should also be considered (Fager, 1957). For group A the stations with four of the six species, for groups B and C stations with three of the four species, for groups D, E, and F stations with two of the three species, and for groups G and H stations containing both species were considered as representing the groups. As a measure of the similarity in distribution of the groups among the stations, the index of Sørensen (1948) was used. The formula for the index is:

$$K_{ij} = \frac{2C}{A + B}$$

where A is the number of stations on which recurrent group *i* occurred; B is the number of stations on which recurrent group *j* occurred; and C is the number of stations on which the two recurrent groups occurred together. The index was calculated for all possible pairs of the eight recurrent groups, and the resulting matrix of the index of similarity was arranged (Table 2). Group E

TABLE 2. Indices of similarity in distribution among the stations of the recurrent groups.

	E	H	F	C	B	D	A	G
E		0.67	0.57	0.50	0.27	0.27	0.29	0.15
H			0.50	0.20	0	0.15	0.11	0
F				0.33	0.27	0.13	0.19	0
C					0.31	0.15	0.21	0
B						0.38	0.27	0
D							0.45	0.14
A								0.40

had the highest sum of similarities with the other groups and was placed first in the matrix. The other groups were arranged according to their degree of similarity, i.e., the matrix was arranged so as to get the highest values of the index along the diagonal of the matrix. The groups E, F, H, and partly C seem to form an assemblage with distinct similarities in distribution among the stations, whereas the recurrent groups B, D, A, and G show high similarity only to their nearest neighbor in the matrix, i.e., they indicate a gradual change in the preference of stations. The groups E, H, and F are found primarily on the stations inside Puget Sound (Table 3). Group C does not indicate any significant pattern in relation to sediment type or area, whereas the other

TABLE 3. Distribution of recurrent groups among the 1967 stations.

		Recurrent groups							
Stations	Mud (%)	E	H	F	C	B	D	A	G
A	16.23	×	×	×					
C	12.54	×	×	×	×				
D	31.67	×	×	×			×		
E	8.33	×	×						
8	67.50			×		×			
2	66.48					×			
16	61.86			×				×	
18	42.51					×			
30	39.61					×	×		
27	39.36						×		
9	36.33	×		×	×	×		×	
10	31.45			×		×			
26	25.32	×			×	×	×	×	
17	24.12				×				
25	21.98							×	
29	21.33					×	×	×	
21	20.99						×	×	
3	20.71		×					×	
5	10.56							×	×
7	6.56						×	×	
15	5.63							×	×
6	5.37							×	
22	5.25				×				
4	4.58							×	
11	4.19							×	×
12	3.58						×	×	×
14	1.45								×
24	0.79	×						×	×

four groups show a distinct trend from stations with a high (group B) to stations with a low (group G) percentage of mud in the sediments. Thus, there is some indication that the recurrent groups are not merely statistical artifacts, but may represent meaningful ecological groupings. Fager (1957) suggested a number of tests of interspecific relations within groups to determine whether the groups had any ecological significance. The tests have been applied to the various recurrent groups (Fig. 3), and the results are discussed below.

Group A consists of six species, which were ranked as 1, 2, 3, 4, 6, and 7 in frequency of occurrence among the 37 stations. Thus, the group seems to represent the species that are widely distributed in the area. However, although these species have wide tolerance ranges in common, they may be distinctly different in choices of the most favorable habitat. Therefore, the coefficient of concordance was very low ($W = 0.07$). There was a certain degree of dominance in the group ($W = 0.23$, $P < 0.01$) and *Ampelisca macrocephala* was the

overwhelmingly dominant species in the group. There was significant correlation in abundance between *Axinopsida sericata* and *Amphiodia urtica* ($\tau = 0.43$, $0.02 < P < 0.05$), but not between any other pair in the group.

Since group A is made up of ubiquitous species, the composition of the group will be strongly dependent upon the sampling design. If more samples had been taken at greater depths where the sediment generally is softer, typical shallow-water sand-bottom species (e.g., *Diastylopsis dawsoni* and *Ampelisca macrocephala*) might have been excluded from the group and soft bottom species (e.g., *Heterophoxus oculatus* and *Macoma carlottensis*) might have been included. Therefore, the group is of very little value for a study of the relations between benthic communities and environmental parameters. However, as the ubiquitous species often are dominating both in numbers and biomass the species of group A may in fact be the most important species for future studies of the productivity of the benthos off the coast of Washington.

Group B showed weak agreement among the species as to what constituted a favorable habitat ($W = 0.31$, $P > 0.20$), but Table 3 shows that the group was found mainly on the stations with soft sediments. There was not significant correlation of abundance between any pair of species in the group, but there were significant dominance relations ($W = 0.55$, $P < 0.01$), with *Heterophoxus oculatus* as the most important, *Amphioplus* sp. as number two, and *Macoma elimata* and *Tellina carpenteri* about equal in importance.

The species of group C were in poor agreement in choice of favorable habitat ($W = 0.36$, $P > 0.20$), and the distribution of the group cannot be explained according to sediment types or depths (Table 3). The group may therefore be related to other environmental parameters that have not been measured in the present study.

The three species in group D did not show significant interrelations when tested by either of the statistical tests, and since the group occurs at stations widely different in sediment types (Table 3), the occurrence of the group is difficult to interpret.

Group E showed significant coefficient of concordance ($W = 0.69$, $0.02 < P < 0.05$), and there was significance between the rankings of abundance of *Diastylis alaskensis* and *Psephidia lordi* ($\tau = 0.83$, $0.01 < P < 0.02$). The three species in the group had been found regularly on shallow-water sand-bottom stations in Puget Sound during 1963–64 (Lie, 1968).

None of the statistical tests revealed significant relationships among the species in group F, but Table 3 shows that the group occurred at stations in Puget Sound and at offshore stations with fairly high mud content.

Group G consisted of the amphipods *Paraphoxus abronius* and *P. obtusidens*, and the rank correlation based on abundances for the two species was high ($\tau = 0.50$, $0.02 < P < 0.05$). The species were particularly abundant at the shallow-water offshore stations with low mud content in the sediments (Table 3), and the two species had mean ranks of 4 and 5 in abundance among the 36 species at the six stations where the group occurred.

The two species in group H showed a rather low rank correlation based on their abundances ($\tau = 0.30$, $0.10 < P < 0.20$). They occurred nearly exclusively at the Puget Sound stations, as did the species of group E (Table 4). The two groups were strongly interconnected (Fig. 3), and during 1963–64 all five species of the two groups showed similar sediment preferences in Puget Sound (Lie, 1968).

In conclusion, the recurrent group analysis applied to the present data resulted in two groups with a distinct geographical distribution (groups E and H), two groups with distinct sediment preferences (B and G), and three groups not explainable according to sediment preference or geographical distribution. One group (group A) was composed of species with ubiquitous distribution in the investigated area and was therefore useless as an indicator group for ecological relations. It is therefore concluded that the recurrent group analysis is only moderately helpful for identification and interpretation of the benthic communities in the present material.

FACTOR ANALYSIS

The Q-Mode

The factor analysis was applied to the 36 most frequently occurring species of the 1967 material for comparison with Fager's recurrent group method and the index of affinity method. Later the 1968 material was added to the 1967 material and the same 36 species were subjected to factor analysis only. However, when the 1968 data were added, *Melita desdichada*, *Diastylis pellucida*, *Nemocardium centifilosum*, and *Amphipholis squamata* occurred less frequently than *Eohaustorius washingtoniensis*, *Hemilamprops californiensis*, *Tellina salmonea*, and *Thyasira gouldii*. The remaining 32 species were still the most frequently occurring, and their rank order remained largely the same ($\tau = 0.67$, $P < 0.01$).

The first three factors in the Q-mode of the 1967 data explained 40.8% of the total variance, but from the fourth factor on, the difference between successive eigenvalues was less than 1% (Fig. 4). The rotation was therefore

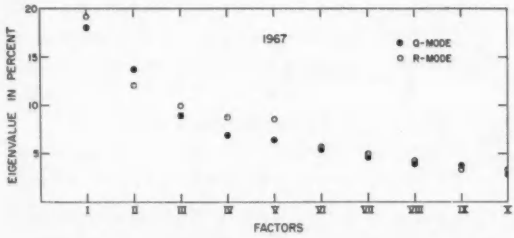


FIG. 4. The 10 highest eigenvalues in the R- and Q-modes of the factor analysis based on the 1967 data.

performed with three factors. A station was considered represented by a factor if the loading was higher than 0.5; however, the stations A and 26 were considered represented by factor I and station 25 by factor II (Fig. 5

and 6). The stations E, F, G, 5, 22, 23, and 28 had particularly low loadings to the three factors based on the 1967 data; these stations, labeled "unclassified" in the figures, had communalities ranging from 0.004 to 0.077. Stations E, F, and G were located in Holmes Harbor (Fig. 1) where the oxygen concentration of the bottom water in autumn is reduced to about 2.1 ml/liter (Barnes and Collias, 1956). Probably as a result of this the fauna was extremely poor, particularly at the two deepest stations, F and G. At the two latter stations the total number of species was 6 and 3 and the number of specimens was 34 and 5, respectively. Station 5 differs from the other stations by the extremely high abundance of the cumacean *Diastylopsis dawsoni* (8505 specimens), and station 23 had a very poor fauna (5 species and 38 specimens). However, stations E, 22, and 28 had no apparent unique features of their fauna or environments that would explain the low loadings to the three factors.

Community I: The Deep-Water Mud-Bottom Community

The 1967 stations characterized by high positive loadings to factor I form a well-defined group (Fig. 5 and 6); only stations 10 and 18 had rather high

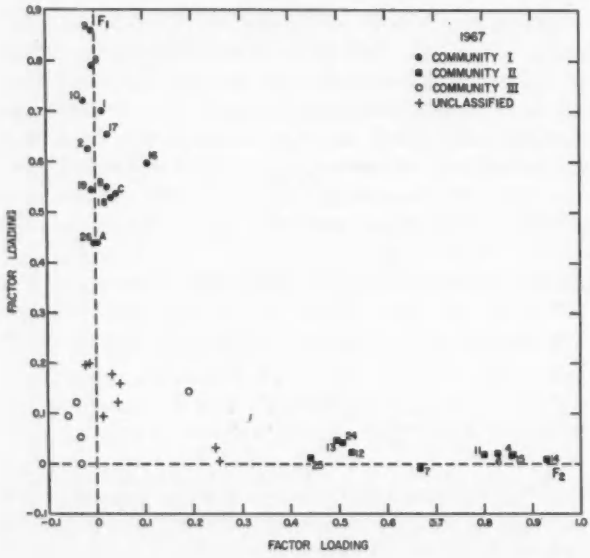


Fig. 5. Two-factor plot of the 1967 data. Rotated factors I and II in the Q-mode.

loadings also to factor III. The factor seems to represent a distinct community distributed in the outer parts of the investigated area off the coast of Washington in depths from 117 to 317 m (mean depth 151 m). Also three stations in Puget Sound off Seattle and one station in Juan de Fuca Strait had rather high loadings to factor I (Fig. 7A).

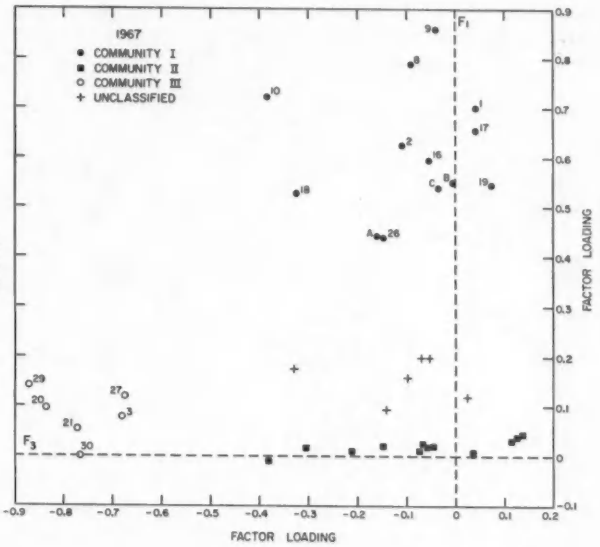


FIG. 6. Two-factor plot of the 1967 data. Rotated factors I and III in the Q-mode.

The factor loadings for some of the 1967 stations changed only slightly when the analysis was performed with the data from 1968 included (Fig. 7B), and the general distribution of the community remained the same.

The stations of the community represented by factor I had generally a bottom type with a high proportion of mud (silt + clay) (Fig. 8). Thus, factor I seems to represent a soft-bottom community from the deeper parts of the continental shelf off the coast of Washington.

COMMUNITY II: THE EXPOSED SHALLOW-WATER SAND-BOTTOM COMMUNITY

Ten of the stations from 1967 had high positive loadings to factor II, and two of them (stations 7 and 11) also had fairly high loadings to factor III (Fig. 5 and 9). The community represented by factor II was distributed in depths from 15 to 83 m (mean depth 45 m) along the coast of Washington and in shallow exposed bays in the outer part of Juan de Fuca Strait (Fig. 10A).

When the analysis included the 1968 stations, nine stations were added to the group. The community represented by the factor was distributed in shallow waters along the entire Washington coast down to the Columbia River (Fig. 10B).

All the stations with high loadings to factor II were characterized by very high percentages of sand in the sediments (Fig. 8). High sand percentages per se may not be of importance for the community, but rather other environmental parameters associated with high sand percentages, such as water turbulence, high amounts of suspended organic matter, etc.

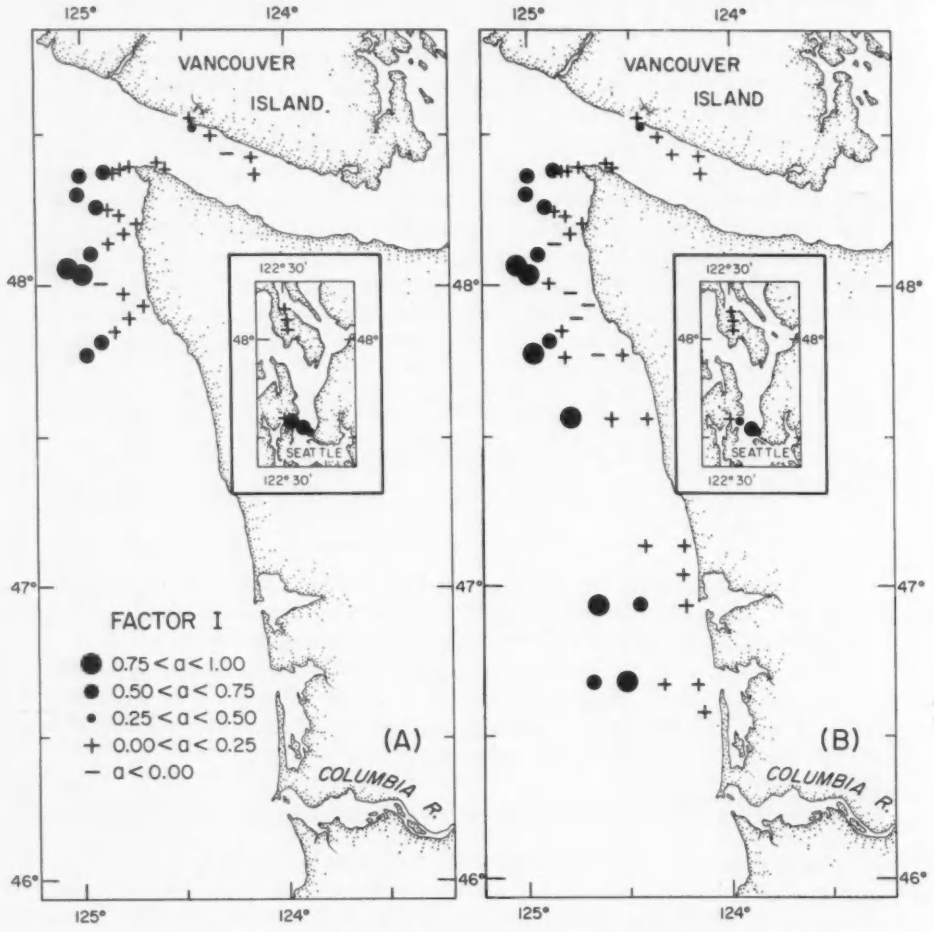


FIG. 7. Quantitative geographic distribution of the loadings to factor I in the Q-mode based on the 1967 data (A) and the combined 1967–68 data (B).

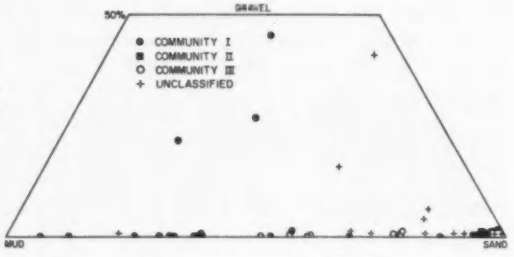


FIG. 8. Position of the three Q-mode communities in a ternary diagram of the percentages of gravel, sand, and mud in the sediments at the stations.

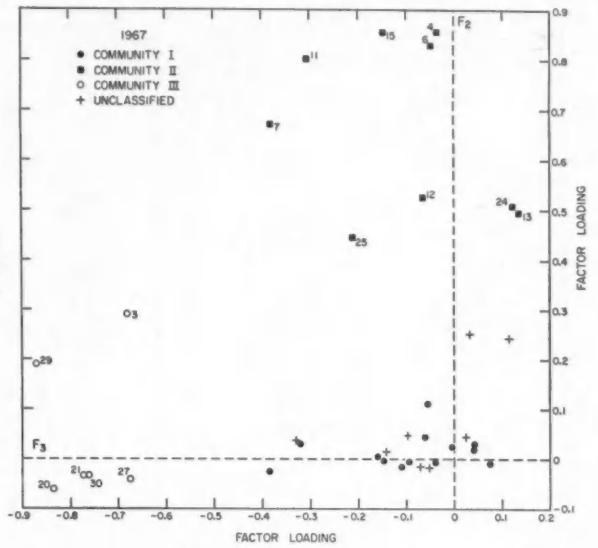


FIG. 9. Two-factor plot of the 1967 data. Rotated factors II and III in the Q-mode.

COMMUNITY III: THE INTERMEDIATE DEPTH SAND-BOTTOM COMMUNITY

The stations with high negative loadings to factor III form a well-defined group, but the factor also influences some of the stations in the first two communities (Fig. 6 and 9). The highest loadings were found on stations in Juan de Fuca Strait, but also some stations in intermediate depths along the coast of Washington had rather high loadings to the factor (Fig. 11A). The mean depth of the stations was 100 m, ranging from 50 to 164 m. The distribution of the community was maintained also when the 1968 data were included in the analysis (Fig. 11B); however, the loadings then changed sign, but since this is a bipolar factor, small changes in the distribution of the data may cause the changes in sign (Harman, 1967).

The community represented by factor III was found on stations with rather high percentages of both mud and sand (Fig. 8); however, the location of the stations in the rotated factor space (Fig. 6 and 9) indicates that factor III represents a distinct community and not merely a transitional zone between the two other communities.

COMPARISON OF THE COMMUNITIES

The ternary diagram representing projections of the stations onto the first octant of the unit sphere (p. 642) shows the relation among the three communities (Fig. 12). Stations 5, 23, 25, 40, and 47, which were unclassified by considering their low loadings to the three factors, are grouped among the community II stations, and this makes sense both in view of their geographic locations (Fig. 1) and their sediment types. Figure 12 leaves the impression

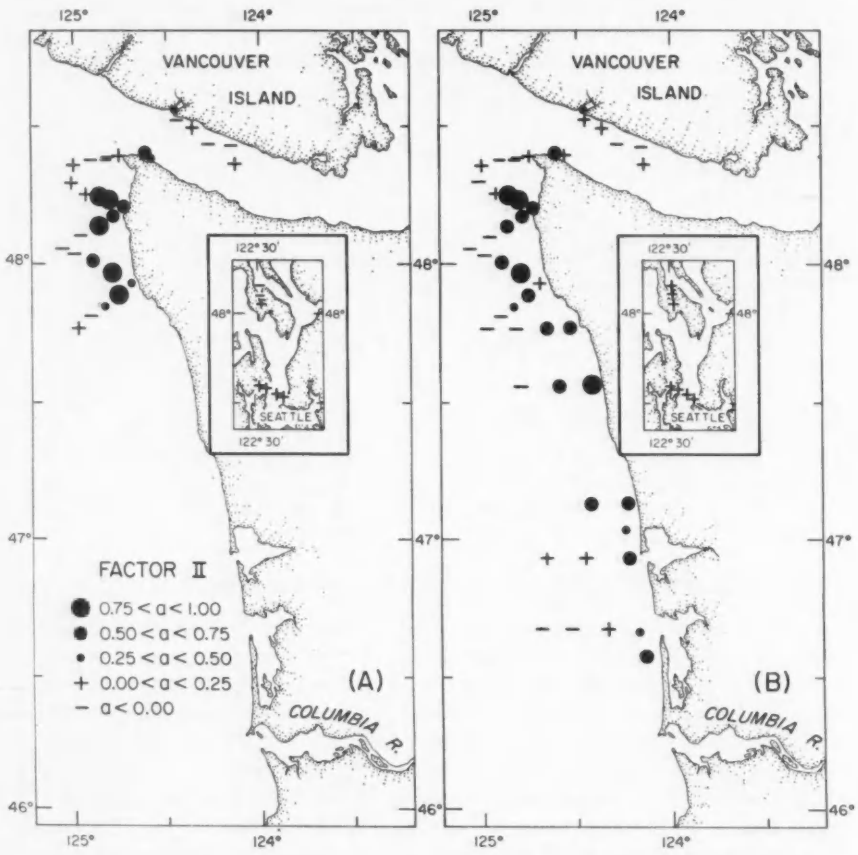


FIG. 10. Quantitative geographic distribution of the loadings to factor II in the Q-mode based on the 1967 data (A) and the combined 1967–68 data (B).

of three discrete communities with no affinity between the communities I and II, but with a certain degree of affinity between community III and the two other communities. Since community III geographically (Fig. 11) and by sediment preference (Fig. 8) is located between communities I and II, the communities of the shelf might also be considered a continuum. Community II is gradually changing into community III and the latter into community I in relation to distance from shore and change in sediment types. However, it must be emphasized that community III is not a transitional zone or ecotone (Hedgpeth, 1957) between the two other communities; this is indicated both by the ordination of the community in the factor space of the Q-mode and by the specific species composition as shown by the R-mode.

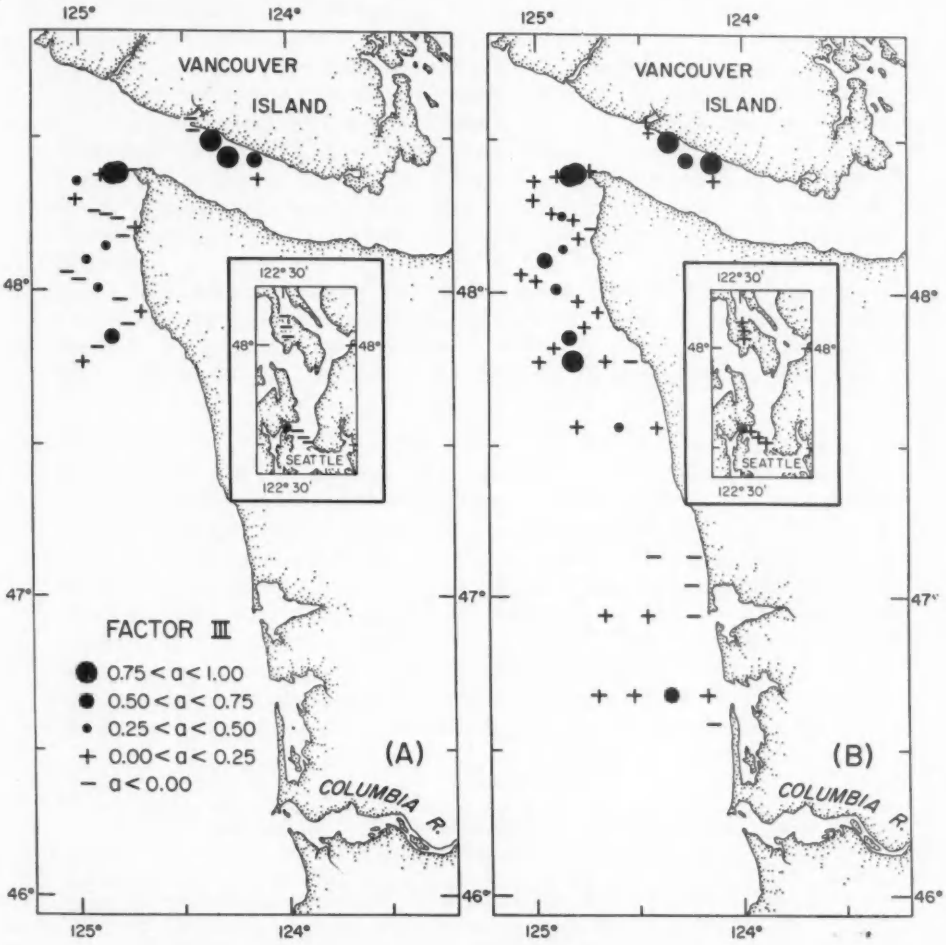


FIG. 11. Quantitative geographic distribution of the loadings to factor III in the Q-mode based on the 1967 data (A) and the combined 1967–68 data (B). For the 1967 data the sign for the loadings is negative, for the combined data the sign is positive.

The communities agree well with groupings based on the relative contributions to total standing crop by the major taxonomic groups in the 1967 material (Lie, 1969). Twelve stations characterized by more than 70% of the weight contributed by polychaetes and echinoderms included 9 of the 10 stations in the community associated with factor I, 11 stations characterized by high weight percentages of both crustaceans and lamellibranchs included 9 of the 10 stations in the community with high loadings to factor II, and 7 stations

FIG. 12. Ternary diagram of the projections of the orientation of the stations in the rotated three-factor space in the Q-mode onto the first octant of the unit sphere.

characterized by high weight percentages of both lamellibranchs and polychaetes included 5 of the 6 stations in the community represented by high negative loadings to factor III. There is, therefore, considerable evidence for the existence of three qualitatively and quantitatively different offshore communities.

THE R-MODE

The first five factors in the R-mode explained 58.7% of the total variance. Between factors V and VI there was a distinct decrease in the percent contribution of the eigenvalues, and from the sixth factor on there was less than 1% difference between successive eigenvalues (Fig. 4). The rotation was therefore performed with the first five extracted factors, and a species was considered represented by a factor if its loading was higher than 0.5.

Table 4 shows the loadings (b_1, b_2, ..., b_5) for the various species to each of the five extracted factors and the communalities over the five factors ($h^2 = \Sigma_j b_j^2$). Six groups of species with high positive or negative loadings to at least one of the factors could be demonstrated, and only *Compsomyax subdiaphana* and *Diastylopsis dawsoni* could not be assigned to any of the six groups. Clearly, this number of groups in relation to the number of stations is too high for meaningful ecological explanation (Dagnelie, 1960), but some of the groups could be identified whether two, three, four, or five factors were extracted. Nearly exactly the same groups resulted from the analysis with the 1968 data included.

The geographic distribution of the species groups and the ecological peculiarities of the species within a group may be used to explain the corresponding underlying factors. Generally, a station is considered representing a group if more than two-thirds of the species in the group occur at the station.

Species Group I

The group is composed of the crustaceans *Euphilomedes producta*, *Westwoodilla caecula*, *Diastylis alaskensis*, and *Pinnixa schmitti*, and the lamellibranchs *Mysella tumida*, *Nucula bellotii*, *Pandora filosa*, and *Psephidia lordi*.

The group was present at the Puget Sound sand-bottom stations (Fig. 13) and at station 46 off the southern part of Washington. The latter station was very rich in species, and three of the other five groups were also represented at the station. In a study from 1963 to 1964, Lie (1968) found that five of the seven species in group I were among the 23 numerically dominant nonpolychaete species at eight permanent benthos stations in Puget Sound. Factor I therefore seems to represent an inshore sand-bottom faunal assemblage.

Species Group II

The group consists of the crustaceans *Paraphoxus abronius* and *Paraphoxus obtusidens*, and the lamellibranch *Tellina buttoni*. The crustacean *Monoculodes spinipes* also had a high loading to factor II (Table 4), but an even higher to factor IV.

The group was distributed on about the same stations that had high loadings to factor II in the Q-mode (Fig. 13). The mean depth at the stations was 32 m (range 15–65 m), and the mean sand percentage of the sediments was 96.63% (95% confidence limits ± 1.63%). The high sand percentages reflect the strong turbulence of the water at these stations, related to the wave action. The three crustaceans mentioned above are particularly well adapted for living in shifting sand as they are rapid burrowers and not tube-dwellers (Barnard, 1961). *Tellina buttoni*, which has been recorded as one of the typical offshore shallow-water species in California (Barnard, 1963) is a selective deposit feeder. The low silt content of the stations where group II occurs suggests that *T. buttoni* must ingest large amounts of inorganic matter to be able to extract a sufficient amount of food.

TABLE 4. Loadings to the first five rotated factors (b_1, b_2, ..., b_5) and the communalities (h^2) for the 36 most frequently occurring species. Species groups I–VI.

Species							
Name	Code no.	b_1	b_2	b_3	b_4	b_5	h^2
Diastylis alaskensis	29	*0.835*	0.012	−0.031	−0.087	−0.216	0.752
Pinnixa schmitti	11	*0.822*	−0.003	0.152	−0.271	−0.043	0.784
Mysella tumida	14	*0.805*	0.061	0.067	0.051	0.124	0.674
Westwoodilla caecula	28	*0.771*	0.041	0.080	−0.157	−0.130	0.644
Euphilomedes producta	27	*0.717*	0.032	−0.361	−0.080	−0.250	0.715
Nucula bellotii	20	*0.661*	−0.393	−0.007	0.146	−0.024	0.613
Pandora filosa	33	*0.654*	0.016	−0.510	−0.093	−0.244	0.756
Psephidia lordi	15	*0.621*	0.031	−0.041	0.435	−0.260	0.645
Paraphoxus abronius	18	−0.102	*0.623*	0.279	0.322	0.160	0.605
Paraphoxus obtusidens	19	−0.112	*0.570*	0.268	0.408	0.094	0.585
Tellina buttoni	25	0.275	*0.465*	0.227	0.264	0.072	0.418
Yoldia ensifera	31	−0.096	*−0.699*	0.132	0.077	−0.027	0.521
Paraphoxus variatus	10	0.156	*−0.688*	0.341	0.023	0.247	0.675
Tellina carpenteri	13	−0.267	*−0.623*	−0.173	0.144	−0.052	0.513
Macoma elimata	7	0.321	*−0.542*	−0.077	0.038	−0.390	0.557
Acila castrensis	22	0.023	*−0.515*	0.335	0.054	−0.372	0.520
Heterophoxus oculatus	4	0.025	−0.134	*−0.787*	−0.123	−0.441	0.847
Macoma carlottensis	8	0.038	0.141	*−0.771*	0.365	0.157	0.773
Adontorhina cyclia	23	−0.043	−0.032	*−0.754*	−0.164	−0.042	0.599
Eudorella pacifica	36	0.135	0.041	*−0.701*	−0.082	0.128	0.535
Amphioplus sp.	16	−0.314	−0.275	*−0.573*	−0.017	−0.086	0.511
Axinopsida sericata	2	0.442	−0.312	*−0.456*	−0.023	0.275	0.577
Diastylis pellucida	35	0.004	−0.133	−0.153	*0.771*	−0.083	0.643
Ampelisca compressa	3	−0.181	0.222	0.225	*0.736*	0.050	0.677
Amphiodia urtica	6	0.032	−0.088	0.235	*0.651*	0.299	0.578
Photis brevipes	5	0.146	−0.408	−0.037	*0.643*	0.158	0.627
Monoculodes spinipes	21	−0.063	0.502	0.134	*0.575*	0.071	0.611
Ampelisca macrocephala	1	−0.141	0.128	−0.134	*0.534*	0.087	0.347
Nuculana minuta	30	0.082	−0.058	−0.017	−0.017	*−0.868*	0.765
Byblis veleronis	9	0.268	0.030	−0.125	−0.032	*−0.828*	0.775
Amphipholis squamata	26	0.025	−0.081	0.147	−0.106	*−0.702*	0.533
Ophiura lütkeni	17	−0.046	−0.101	−0.214	−0.182	*−0.577*	0.424
Melita desdichada	34	0.222	−0.226	0.229	−0.038	*−0.526*	0.430
Nemocardium centifilosum	32	0.206	−0.055	−0.395	0.048	*−0.515*	0.469
Compsomyax subdiaphana	24	0.062	−0.155	−0.136	−0.400	0.347	0.327
Diastylopsis dawsoni	12	−0.176	0.228	0.052	0.107	0.150	0.120

I II III IV V VI

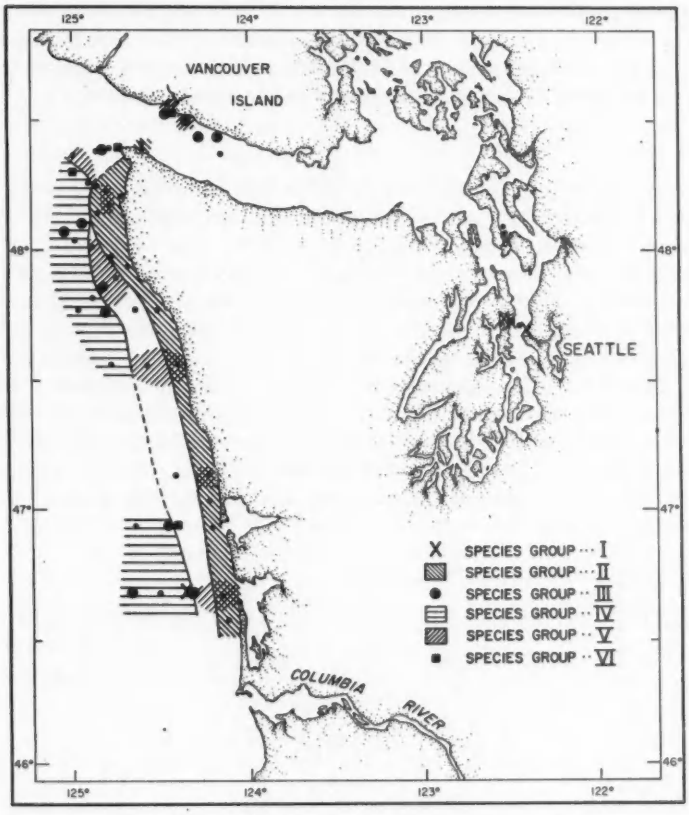


FIG. 13. Geographic distribution of the species groups determined by the R-mode of the factor analysis.

High positive loading to factor II represents a faunal element adapted to shallow waters off exposed oceanic beaches along the coast of Washington.

SPECIES GROUP III

The group consisting of the amphipod *Paraphoxus variatus* and the lamellibranchs *Tellina carpenteri*, *Macoma elimata*, *Yoldia ensifera*, and *Acila castrensis* was characterized by high negative loadings to factor II (Table 4).

The group was found on 12 stations (Fig. 13), among which were included seven of the eight stations with high loadings to factor III in the Q-mode (Fig. 11B). The stations were distributed in depths from 50 to 180 m (mean 108 m), and the sediments at the stations were characterized as sand or silty sand, with mean sand proportion 60.76 ± 13.29%. The lamellibranchs in the group are classified as selective deposit feeders (Semenov, 1965), and other lamellibranchs

of the same feeding-type, such as *Nucula bellotii* and *Axinopsida sericata*, also have relatively high negative loadings to factor II (Table 4). Species group III therefore seems to represent a faunal assemblage on deep-water sand bottoms dominated by selective deposit feeding lamellibranchs.

Species Group IV

The group defined by high negative loadings to factor III consists of the amphipod *Heterophoxus oculatus*, the cumacean *Eudorella pacifica*, the lamellibranchs *Adontorhina cyclia*, *Axinopsida sericata*, and *Macoma carlottensis*, and the ophiuroid *Amphioplus* sp. (Table 4). The group was found on 16 stations (Fig. 13), among which were included 14 of the 18 stations with high loadings to factor I in the Q-mode (Fig. 7B). The stations were distributed in depths from 26 to 207 m (mean 120 m) in muddy sediments (mean percentage silt + clay 50.44 $\pm$ 12.33%). *Heterophoxus oculatus*, *Eudorella pacifica*, *Axinopsida sericata*, and *Macoma carlottensis* had been among the numerically dominant species in Puget Sound in 1963–64 (Lie, 1968), with their highest abundances at the stations with sediments dominated by mud and muddy sand. Species group IV therefore seems to represent the soft bottom community along the coast of Washington.

Species Group V

The group defined by high positive loadings to factor IV consists of the amphipods *Ampelisca compressa*, *Ampelisca macrocephala*, *Monoculodes spinipes*, and *Photis brevipes*, the cumacean *Diastylis pellucida*, and the ophiuroid *Amphiodia urtica* (Table 4). The group was found on 15 stations (Fig. 13), among which were 10 of the 18 stations with high loadings to factor II in the Q-mode (Fig. 10B). Species group V thus has a distribution rather similar to species group II, and the species in group II have also rather high loadings to factor IV (Table 4). However, group V was found in deeper water than group II (mean depth 72 vs. 32 m), and in somewhat muddier sediments (mean sand percentage 83.72 vs. 96.99). The difference in sediment types was statistically significant when tested by a *t*-test ($0.01 < P < 0.05$).

The species in the group were among the most widely distributed of the species; they were ranked as 1, 3, 5, 6, 21, and 36 in frequency of occurrence on the 55 stations.

Species group V represented by high loadings to factor IV seems to represent the fauna of the deepest parts of the shallow-water sand-bottom community off the coast of Washington. The group considerably overlaps in its distribution species group II and is therefore not considered representing a separate community.

Species Group VI

The group characterized by high negative loadings to factor V consists of the amphipods *Byblis veleronis* and *Melita desdichada*, the lamellibranchs

Nemocardium centifilosum and *Nuculana minuta*, and the ophiuroids *Ophiura lütkeni* and *Amphipholis squamata* (Table 4).

The group was found on five stations (Fig. 13) in depths from 26 to 155 m (mean 95 m), and the stations were characterized by high amounts of gravel. The five stations were ranked as 1, 2, 3, 6, and 7 in gravel percentage among the 55 stations. The same species had been abundant at stations dominated by gravel or coarse sand in Puget Sound during 1963–64 (Lie, 1968). The group therefore seems to represent the fauna of coarse bottom types.

DISCUSSION

The index of affinity gave a grouping of stations that could be explained according to bottom types (Fig. 2A), and the groups of stations correspond to similar groupings based on the Q-mode of the factor analysis (Fig. 7, 10, 11). The first group of the index of affinity (stations F–C) consists of stations with either no high loadings to any of the factors or with high loadings to factor I; the stations of the second group (stations 10–9) have all high loadings to factor I; those of the third group (stations 6–23) have high loadings to factor II (except stations 5 and 23); and six of the eight stations in the fourth group (stations 21–26) have high loadings to factor III. This good agreement between the two techniques is surprising in view of the fact that the index of affinity is based on the presence and absence of all the 127 species, whereas the factor analysis is based on the abundances of only the 36 most frequently occurring species.

In spite of the similarity, the factor analysis seems preferable to the index of affinity analysis because important information about the communities is lost when the species abundances are neglected. Furthermore, the relations among the stations are easier to understand in a space of a small number of orthogonal factors (Fig. 5, 6, and 9) than in a trellis-diagram (Fig. 2). On the other hand, the index of affinity has the advantage of including all the species, and can be applied when enumeration is impossible (as in epifaunal groups like hydroids, bryozoans, etc.). However, Dagnelie (1960) has shown that factor analysis can also be applied to presence/absence data.

A major advantage of a factor analytical treatment of synecological data is the possibility of a quantitative assessment of the underlying causes for the distribution of the communities. Since the factors are orthogonal, they can be considered dependent variables and the various environmental parameters independent variables, and thus through multiple regression the relations among the factors and the environmental parameters may be determined quantitatively (Draper and Smith, 1966). For a reasonable explanation of the causality, the appropriate biotic and abiotic environmental parameters must be measured. In the present material only the particle size of the sediments and the depths were measured, but one might expect that other parameters such as physical and chemical parameters of the water, sediment chemistry, quantity and type of food available, competition and predation will also determine the composition and distribution of the communities. The complexity

of the situation is probably reflected in the relatively low concentration of variance in the first factors (Fig. 4), but the various communities did show a rather distinct geographical distribution and could be identified with definite depths or bottom types. Thus by focusing the attention on only three variables and by suggesting possible causal relations, the role of the factor analysis in the present situation has been hypothesis-creating rather than hypothesis-testing (Cattell, 1965a, b).

The species groups as determined by Fager's recurrent group analysis also showed a certain agreement with groupings based on the R-mode of the factor analysis (Fig. 14). Recurrent groups E and H are composed of species

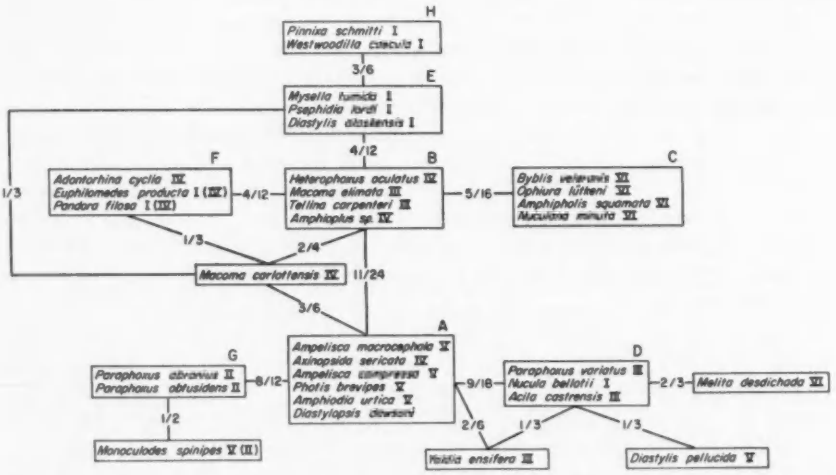


FIG. 14. Classification of the species into recurrent groups (A–H) and species groups determined by the R-mode of the factor analysis (I–VI).

from species group I only, and recurrent group C of species from species group VI. Recurrent group G has species from species group II; the associated species *Monoculodes spinipes* is in species group V, but it also has a rather high loading (0.502) to factor II. Similarly, *Nucula bellotii* in recurrent group D has a loading of −0.393 to factor II, which characterizes species group III, and thus there is good agreement between recurrent group D and species group III. Four of the six species in recurrent group A were from species group V.

The good agreement between the two methods may be related to the high number of zero values in the abundances of the species on the stations (Table 1). In such instances presence/absence yields nearly as much information as abundance, whereas when the species are distributed over most of the stations the two techniques may give significantly different results. The two most widely distributed species, *Ampelisca macrocephala* and *Axinopsida sericata*,

were grouped together by Fager's analysis although their choice of favorable substrate was distinctly different, whereas the factor analysis was able to separate the two species. Thus, factor analysis is preferable on data from small areas where more or less continuous distribution may be expected for a considerable number of species (Greig-Smith, 1964).

The recurrent group analysis and the factor analysis yield results that are basically different, the former being based on qualitative data (presence/absence of species) and the latter on quantitative data (abundance of species). The factor analysis will therefore be better suited for explaining the causes for similarity in species distributions, particularly if a significant part of the total variance could be explained by a small number of factors. The first five eigenvalues in the R-mode included 58.7% of the total variance in the 1967 data, and 56.1% in the combined data from 1967 to 1968. This is a somewhat lower concentration of the variance in the first eigenvalues than reported for similar data from plant and animal ecology. In terrestrial plant communities Goodall (1954) found 72% of the variance in the first four eigenvalues, and in marine zooplankton communities Colebrook (1964) found 71.3% in the first four eigenvalues. Both studies were principal component analyses. In a study of fossil ostracods from boreholes, Reyment (1963) found 57% of the variance in the first four eigenvalues of a principal component analysis, and 66% in a factor analysis of the same data. Rather high proportions of unexplained variance must be expected in complex animal communities partly as a result of sampling variability and partly because the success of a species is determined not only by the environmental effects represented by the common factors, but also to some extent by specific factors that do not affect all the species. Such specific factors may represent interspecific ecological relations such as predation or competition for food or space for certain species.

The communality for the five factors was higher than 0.5 for 29 (80.6%) of the 36 species, i.e., for these species the factors represented more than 50% of their total variance. These "differential species" (Dagnelie, 1960) will yield the most information about the nature of the common factors, and should therefore be awarded particular attention in future studies. Thus, by focusing attention on the "differential species," by ordinating the various species in the factor space, and by suggesting an interpretation of the factors with respect to environmental parameters, the factor analysis in the R-mode also has served mainly as a hypothesis-creating technique.

ACKNOWLEDGMENTS

The present investigation was supported by the U.S. Atomic Energy Commission, contract AT(45-1)-1725 (ref. RLO-1725-164), principal investigator Dr C. A. Barnes. This is contribution 532 from the Department of Oceanography, University of Washington, Seattle, Wash.

We thank Dr E. W. Fager for his help in performing the recurrent group analysis, and Mr D. S. Kisker for identification of the lamellibranchs. Thanks are also due to Drs K. Banse and B. W. Frost for criticism of the manuscript.

REFERENCES

ANDERSON, T. W. 1958. An introduction to multivariate statistical analysis. John Wiley and Sons, New York. 374 p.

BARNARD, J. L. 1961. Relationship of Californian amphipod faunas in Newport Bay and the open sea. Pac. Natur. 2: 166–186.
1963. Relationship of benthic Amphipoda to invertebrate communities of inshore sublittoral sands of Southern California. Pac. Natur. 3: 439–467.

BARNES, C. A., AND E. E. COLLIAS. 1956. Physical and chemical data, Puget Sound and approaches, January–December 1953. Univ. Wash. Dep. Oceanogr. Tech. Rep. 45: 212 p.

BARTLETT, M. S. 1947. The use of transformations. Biometrics 3: 39–52.

CASSIE, R. M., AND A. D. MICHAEL. 1968. Fauna and sediments of an intertidal mudflat: a multivariate analysis. J. Exp. Mar. Biol. Ecol. 2: 1–23.

CATTELL, R. B. 1965a. Factor analysis: an introduction to essentials. I. The purpose and the underlying models. Biometrics 21: 190–215.
1965b. Factor analysis: an introduction to essentials. II. The role of factor analysis in research. Biometrics 21: 405–435.

COLEBROOK, J. M. 1964. Continuous plankton records: a principal component analysis of the geographical distribution of zooplankton. Bull. Mar. Ecol. 6: 78–100.

CREAGER, J. S., D. A. MCMANUS, AND E. E. COLLIAS. 1962. Electronic data processing in sedimentary size analyses. J. Sediment. Petrology 32: 833–839.

DAGNELIE, P. 1960. Contribution a l'étude des communautés végétales par l'analyse factorielle. Bull. Serv. Carte Phytogeogr. B 5: 7–71 and 93–195.
1965. L'étude des communautés végétales par l'analyse statistique des liaisons entre les espèces et les variables écologiques: Principes fondamentaux. Un exemple. Biometrics 21: 345–361 and 890–907.

DRAPER, N. R., AND H. SMITH. 1966. Applied regression analysis. John Wiley and Sons, New York. 407 p.

FAGER, E. W. 1957. Determination and analysis of recurrent groups. Ecology 33: 586–596.
1968. The community of invertebrates in decaying oak wood. J. Anim. Ecol. 37: 121–142.

FAGER, E. W., AND A. R. LONGHURST. 1968. Recurrent group analysis of species assemblages of demersal fish in the Gulf of Guinea. J. Fish. Res. Bd Canada 25: 1405–1421.

FAGER, E. W., AND J. A. MCGOWAN. 1963. Zooplankton species groups in the North Pacific. Science 140: 453–460.

GLEASON, H. A. 1926. The individualistic concept of the plant association. Bull. Torrey Bot. Club 53: 7–26.

GOODALL, D. W. 1954. Objective methods for the classification of vegetation. III. An essay in the use of factor analysis. Aust. J. Bot. 2: 304–324.

GREIG-SMITH, P. 1964. Quantitative plant ecology. 2nd ed. Butterworths, London. 254 p.

HARMAN, H. H. 1967. Modern factor analysis. 2nd ed. Univ. Chicago Press, Chicago, Ill. 469 p.

HEDGPETH, J. W. 1957. Concepts in marine ecology. *In* J. W. Hedgpeth (ed.] Treatise on marine ecology and paleoecology, Vol. 1. Geol. Soc. Amer. Mem. 67: 29–52.

IMBRIE, J., AND T. H. VAN ANDEL. 1964. Vector analysis of heavy-mineral data. Geol. Soc. Amer. Bull. 75: 1131–1156.

JONES, N. S. 1950. Bottom fauna communities. Biol. Rev. 25: 283–313.

KAISER, H. F. 1958. The varimax criterion for analytic rotation in factor analysis. Psychometrika 23: 187–200.

KENDALL, M. G. 1957. A course in multivariate analysis. Charles Griffin & Co., London. 185 p.
1962. Rank correlation methods. 3rd ed. Hafner Publ. Co., New York. 199 p.

KRUMBEIN, W. C., AND F. J. PETTIJOHN. 1938. Manual of sedimentary petrography. Appleton-Century-Crofts, Inc., New York. 549 p.

LIE, U. 1968. A quantitative study of benthic infauna in Puget Sound, Washington, U.S.A., in 1963–1964. Fiskeridir. Skr. Ser. Havunders. 14: 229–556.
1969. Standing crop of benthic infauna in Puget Sound and off the coast of Washington. J. Fish. Res. Bd Canada 26: 55–62.

LINDROTH, A. 1935. Die Associationen der marinen Weichboden. Zool. Bidrag. Uppsala 15: 331–367.

LOOMAN, J., AND J. B. CAMPBELL. 1960. Adaptation of Sørensen's K (1948) for estimating unit affinities in prairie vegetation. Ecology 41: 409–416.

MACFADYEN, A. 1963. Animal ecology, aims and methods. 2nd ed. Sir Isaac Pitman & Sons Ltd., London. 344 p.

MOLANDER, A. 1928. Animal communities on soft bottom areas in the Gullmar Fjord. Kristinebergs Zool. Sta. 1877–1927 2: 1–90.

NICHOLS, F. H. 1970. Benthic polychaete assemblages and their relationship to the sediment in Port Madison, Washington. Mar. Biol. (In press.)

PETERSEN, C. G. J. 1913. Valuation of the sea. II. The animal communities of the sea bottom and their importance for marine zoogeography. Rep. Dan. Biol. Sta. 21: 1–44.
1918. The sea bottom and its production of fish food. Rep. Dan. Biol. Sta. 25: 1–62.

REYMENT, R. A. 1963. Multivariate analytical treatment of quantitative species associations: an example from palaeoecology. J. Anim. Ecol. 32: 535–547.

SEMENOV, V. N. 1965. Quantitative distribution of benthic fauna of the shelf and upper part of the slope in the Gulf of Alaska. Tr. VNIRO 58: 49–77. (Transl. from Russian by the Israel Program for Sci. Transl., U.S. Dep. Com., Washington, D.C., No. TT 67-51206: 37–69, 1968)

SHEARD, K. 1965. Species groups in the zooplankton of eastern Australian slope waters. Aust. J. Mar. Freshwater Res. 16: 219–254.

SØRENSEN, T. 1948. A method of stabilizing groups of equivalent amplitude in plant sociology based on the similarity of species content and its application to analyses of the vegetation on Danish commons. Biol. Skr. 5(4): 1–34.

THORSON, G. 1957. Bottom communities (sublittoral and shallow shelf). *In* J. W. Hedgpeth [ed.] Treatise on marine ecology and palaeoecology, Vol. 1. Geol. Soc. Amer. Mem. 67: 461–534.

WHITTAKER, R. H. 1967. Gradient analysis of vegetation. Biol. Rev. 42: 207–264.

WILLIAMSON, M. H. 1961. An ecological survey of a Scottish herring fishery. Part IV: Changes in the plankton during the period 1949 to 1959. With appendix: A method for studying the relation of plankton variations to hydrography. Bull. Mar. Ecol. 5: 207–229.
1963. The relation of plankton to some parameters of the herring population of the northwestern North Sea. Rapp. Proces-Verbaux Reunions Cons. Perma. Int. Explor. Mer 154: 179–185.

Movement of Phosphate and Other Ions From and Through Lake Muds

H. B. N. Hynes and B. J. Greib

Department of Biology
University of Waterloo, Waterloo, Ont.

Hynes, H. B. N., and B. J. Greib. 1970. Movement of phosphate and other ions from and through lake muds. J. Fish. Res. Bd. Canada 27: 653–668.

Laboratory experiments showed that sheets of polyethylene or a layer of sand placed on the surface of undisturbed lake mud reduce the loss of oxygen in the overlying water and greatly decrease the rate of release of phosphate, iron, and ammonium from the sediment. Polyethylene laid on disturbed mud produced the same effects, and it also reduced the rate of release of total ions as measured by electrical conductivity.

Experiments with P^{32} showed that phosphate moves readily through undisturbed mud, and that the movement is not a biotic process. We also found that phosphate and ammonium injected 4 cm below the mud–water interface move upwards into the water, and that this applies to both a rich calcareous mud and a poor acid peaty one.

We concluded that early reports of little movement of phosphate through muds are erroneous, possibly because they are based on short experiments, and that there is a great potential store of nutrient salts in enriched muds. This would greatly delay recovery of lakes that have been rendered eutrophic by pollution, so we suggest that polyethylene sheet laid on the sediments of valued lakes may serve to rehabilitate them more rapidly.

Hynes, H. B. N., and B. J. Greib. 1970. Movement of phosphate and other ions from and through lake muds. J. Fish. Res. Res. Canada 27: 653–668.

On a démontré en laboratoire qu'en étalant des feuilles de polythène ou une couche de sable sur de la vase non agitée, prélevée au fond d'un lac, il est possible de reduire la perte d'oxygène dans l'eau sus-jacente et de diminuer considérablement la vitesse à laquelle le sédiment relâche les ions de phosphate, de fer et d'ammonium. Ces conclusions s'avèrent exactes même dans le cas où on a utilisé des feuilles de polythène sur un fond de vase préalablement soumis à un brassage; dans ce cas, on a observé aussi que le polythène réduit la vitesse de diffusion de tous les ions, telle que mesurée par conductibilité électrique. L'utilisation du P^{32} a permis de démontrer que le phosphate se déplace aisément dans de la vase non agitée et qu'il ne s'agit pas là d'un processus biologique. On a constaté aussi que des ions de phosphate et d'ammonium injectés 4 cm au-dessous de la jonction eau-vase se déplacent vers le haut et sont libérés dans l'eau, et ce, qu'il s'agisse de vase calcaire riche ou de vase tourbeuse et acide. Les conclusions antérieures voulant qu'il y ait peu de movement d'ions dans la vase s'avèrent fausses, probablement parce que basées sur des expériences de trop courte durée; au contraire, la vase enrichie peut constituer un réservoir très important de sels nutritifs, ce qui retarde considérablement le recouvrement de lacs devenues eutrophes à la suite de la pollution; c'est pourquoi, nous suggérons que certains lacs de valeur pourraient être récupérés plus rapidement si l'on étendait des feuilles de polythène sur les sédiments.

Received October 30, 1969

Printed in Canada (J1637)

INTRODUCTION

IN A PREVIOUS PAPER we showed that a sheet of polyethylene or a thin layer of sand placed over lake muds under deoxygenated water in the laboratory markedly reduces the rate at which phosphate and some other unidentified ions are released from the sediment (Hynes and Greib, 1967). We also suggested that this might provide a method of rendering small and valued lakes less eutrophic. However, as the experiments had been conducted on disturbed mud, and so might not reflect the natural situation, and in view of certain anomalies in the literature that are outlined below, we extended our studies to include study of undisturbed mud and the movement of nutrient ions through it.

It is generally accepted that eutrophication and its attendant problems result from increases in phosphate, ionic nitrogen, iron, and some other elements, but that phosphate is the most important (Ohle, 1953; Mackenthun, 1968). It is also known that lake muds contain much higher concentrations of phosphate than does the water (Olsen, 1958a; Holden, 1961; Hepher, 1966), and that there are large amounts of nitrogen in sediments (Juday et al., 1941). The phosphate in the mud appears to be in dynamic equilibrium with that in the water, as has been found in lakes and fish ponds (Hayes et al., 1952; Olsen, 1958a; Hepher, 1966), and laboratory experiments have shown that the level of this equilibrium depends upon a number of factors. These include oxygen tension, redox potential, *p*H, and the relative iron concentration (Olsen, 1958a, b, 1964; Macpherson et al., 1958), but the net result is that, for any particular set of conditions, sediments, especially if they are agitated, maintain a fairly constant, even though low, concentration of phosphate in the water (Pomeroy et al., 1965). The rate of release may be lower than the potential rate of use of the ion by the phytoplankton (Hepher, 1966), and once it reaches the water, phosphate is rapidly taken up by organisms so that very little remains as simple orthophosphate (Hutchinson, 1957; Rigler, 1956, 1964, 1968).

One result of the release of ions from the mud is that under reducing conditions there is a higher concentration of phosphate, and of iron, just above the mud–water interface than in the overlying water (Hayes, 1964; Sullivan, 1967), a possibility that was also suggested by Hutchinson (1957). And one result of factors controlling the level of the equilibrium is that in calcareous waters, or upon the addition of calcium carbonate, phosphate tends to be precipitated; the equilibrium is towards the mud phase (Gessner, 1939; Zicker et al., 1956; Hepher, 1958). It has also been shown, by working with sterilized mud, that the equilibrium is not dependent upon microorganisms (Macpherson et al., 1958; Pomeroy et al., 1965), although Hayes (1955; Hayes and Phillips, 1958), using antibiotics, demonstrated that bacteria do exert some influence under aerobic as opposed to anaerobic conditions.

The above points seem to be generally accepted, but disagreements arise as to how great a depth of the mud is involved. Most of the laboratory work has been done with disturbed mud, or with agitated mixtures of mud and

water, so it provides no answer to this question. Hayes et al. (1952) calculated that only the upper 2 cm of the sediment was involved in the uptake of P^{32} added to a lake, and Hayes (1955) in experiments with undisturbed cores found that P^{32} added to the overlying water had penetrated only into the top millimeter of mud after 2 weeks. Similarly Zicker et al. (1965) found that little P^{32} was released from radioactive superphosphate fertilizer placed $\frac{1}{4}$ inch (6 mm) below the mud surface after 15 days, and none from 1 inch (25 mm) down. On the other hand Holden (1961) found evidence of deeper penetration of phosphate added to a lake, and suggested that the upper 15 cm of the mud are involved. Also Proft (1967) has demonstrated that the phosphate content of the mud of the Stechlinsee varies with the distance below the mud–water interface, and that the pattern of this variation is different in the littoral and profundal parts of the lake. This seems to point to some dynamic equilibrium of movement through the mud.

The question of how much of the mud forms part of the system is clearly of great importance in the control of eutrophication. If only a thin layer is involved the condition must, as Hayes et al. (1952) suggest, depend upon the constant addition of phosphate. This is almost certainly not so if only because the upper few centimetres are involved in animal activities (Hayes, 1964); but if great depths of mud are involved the recovery of a lake from severe eutrophication caused by pollution must be a prolonged process, unless some means can be found of locking the accumulated phosphate into the mud, or of removing the sediment altogether as has been done from some small lakes in Michigan. Pomeroy et al. (1965) were able to wash out about 5 μmoles of P/gram from the mud from an estuary, and similar yields could be expected from many lake sediments (Juday et al., 1941). Our work therefore included study of the movement of phosphate and ionic nitrogen through undisturbed cores of mud.

MATERIALS AND GENERAL METHODS

Most of the mud used in our experiments came from Paradise Lake, a small eutrophic holomictic lake near Waterloo, Ontario, and it was collected from a depth of about 8 m in the deepest part of the lake. It was a typical dark-coloured gyttja at the top, but at a depth of about 10–12 cm there was an abrupt colour change. This may have represented the boundary between Naumann's "actual" and "historical" layers as discussed by Hayes (1964). The division was so sharp, however, that it seems more probable that it had been produced by human activity as the lake is now surrounded by holiday cottages and children's camps.

The mud for one experiment came from Cranberry Bog near Galt, Ont., a small shallow lake in the middle of a large acid bog. The mud was a typical soft peaty dy.

For one experiment the mud was collected with an Ekman grab and was mixed before use; for the others it was taken with a Gilson corer, manufactured by The Freshwater Biological Association, Ambleside, England. This consists of a Plexiglas tube 8.2 cm wide with a lumen 5.3 cm in diameter that is forced vertically into the mud by lead weights. As it descends, water can leave through holes in a screw cap at the upper end, but the resistance of the penetration into the mud releases a trip that closes the holes, and the core can then be withdrawn intact. By adjusting the amount of lead the level of the mud–water interface can be to some extent controlled but we usually arranged that it was high in the tube, and we later adjusted it downwards as described below.

Our tubes were 53.1 cm long and we stoppered them with rubber bungs in the field, excluding all bubbles, and transported them carefully to the laboratory in an upright position. There, by removal of the bottom bung and slow admission of air through a cock in the upper bung or via a spout (see Fig. 1), the mud–water interface was lowered to the required level. The bottom was then restoppered and the water level was made up with lake water. As far as

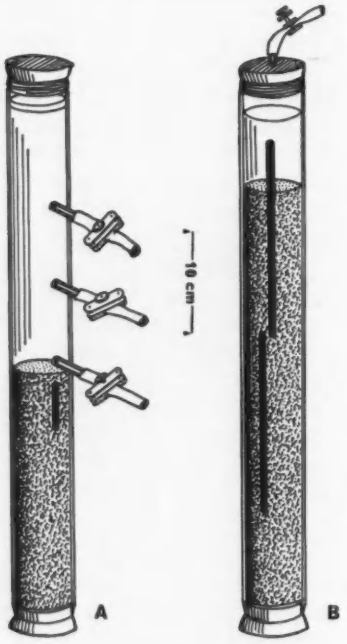


FIG. 1. Two of the four types of tube used in the experiments on undisturbed cores. A, type 2; B, type 3.

could be seen none of these activities disturbed the relationships of the layers of sediment, and we believe that we were working throughout with virtually undisturbed cores.

Our tubes were modified in various ways. Type 1 had 3 spouts with clamps inserted at 12.6, 20.2, and 27.8 cm from the top; in type 2 a slot was cut 2.5 cm below the lowest spout (Fig. 1A). The slots were 3.7 cm long and 0.3 cm wide and were filled with rubber held into place by rubber cement. Type 3 had no spouts but had two overlapping rubber-filled slots 17.5 and 17.8 cm long (Fig. 1B); in type 4 there were single rubber-filled slots 23.2 cm long centered in the length of the tube. The slots in type 3 were staggered to avoid possible splitting of the tube. The spouts allowed controlled extraction and addition of water, and the rubber-filled slots permitted the insertion of hypodermic needles. Tubes of types 3 and 4, which had no spouts, were fitted with a clamped tube through the upper bung (Fig. 1B) to permit pressure equalization when material was inserted or withdrawn by hypodermic syringe.

Analyses were made by the following techniques. Measurement of *p*H was by glass electrode. Phosphate was analyzed by Atkin's modification of Denige's method as described by Mackereth (1963), except that the optical density was measured with a spectrophotometer. Nitrate was measured by the phenoldisulphonic acid method as described by Mackereth (1963) and also spectrophotometrically. Ammonium nitrogen was analyzed by Nessler's method as described in *Standard Methods* (American Public Health Association, 1965). Iron was measured spectrophotometrically by the 2,4,6-tripyridil-*s*-triazine method and oxygen by the azide method and titrated with phenylarsine oxide, Hach Chemical Co. reagents being used for both elements.

RELEASE OF IONS FROM MUD

EXPERIMENT 1

Our previous study had shown that a layer of polyethylene sheet impedes the release of phosphate from the mud surface. The object of this experiment was to determine how effectively complete enclosure in polyethylene sheet prevents the escape of ions.

Lengths of polyethylene tubing, 16 cm in diameter and 0.056 mm thick, were inserted into the necks of four wide-mouthed flasks so that their open ends protruded. Into each tube was poured 200 ml of a solution of phosphate, nitrate, ferrous iron, or ammonia containing about 5 mg/liter of P, N, Fe, and N respectively, and into each flask, outside the tube, was poured 450 ml of distilled water. This resulted in a contact area of about 450 cm^2. The flasks were stoppered with the ends of the tubes outside, and were covered and held at room temperature in the dark.

After 27 and 71 days samples of the outer water were withdrawn for analysis with the following results in milligrams/liter.

	PO_4-P	NO_3-N	Fe^{++}	NH_3-N
27 days	0.000	0.00	0.01	0.21
71 days	Trace	Trace	0.00	0.21

It is clear, therefore, that some ammonia can pass through polyethylene sheet but that phosphate, nitrate, and ferrous iron hardly do so.

EXPERIMENT 2

This experiment was designed to determine the effect of covering undisturbed mud with polyethylene sheet, or with sand, on the release of phosphate, iron, and ammonium.

Fifteen cores were collected in type 1 tubes from Paradise Lake on May 10, 1967, and the mud columns were lowered so that the interfaces were about 3 cm below the lower spout, and there was 28 cm of overlying water.

At this time the conductivity of the water was 210 μmho, *p*H was 8.13, and concentrations of nitrate and ammonium nitrogen were 0.10 and 0.37 mg/liter; there was 10.0 mg/liter of oxygen and only traces of phosphate and iron.

Of the 15 tubes, 5 served as controls, 5 had mud covered with a 1.5-cm layer of coarse acid-cleaned sand, and 5 had mud covered with a close-fitting disc of polyethylene, 0.01 mm thick, attached with rubber cement to a thin glass ring that served as a weight to hold the disc in place on the mud surface.

The tubes were stoppered and kept at 7 C in the dark, and for this experiment, as in our earlier ones (Hynes and Greib, 1967), the surface of the water was covered with a 1.7-cm layer of paraffin oil. This was done to exclude oxygen, but in an experiment run concurrently with this one, in which we covered boiled water in a beaker with oil and measured the rate of entry of

oxygen, we discovered that, in fact, a layer of oil 1 cm thick and 80 cm^2 in area over 900 ml of water only slightly reduced the uptake of oxygen at room temperature. There was a fairly rapid rise of oxygen content in both beakers and after 90 hr 6.85 mg/liter was present below the oil as compared with 7.90 in the control. We therefore abandoned oil in later experiments, and relied on the bungs and the stillness of the water to allow the lower layers to deoxygenate.

At intervals, as shown in Fig. 2, water was drawn from the lower spout for analysis, and the amount drawn off was replaced immediately by slow addition through the upper spout of filtered lake water at 7 C that had been previously deoxygenated by boiling.

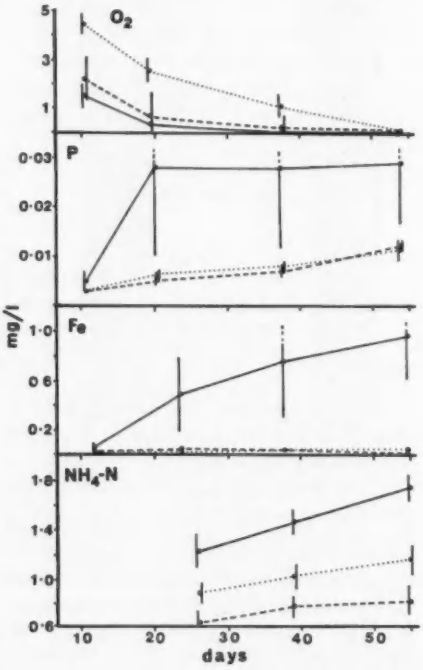


FIG. 2. Concentrations of oxygen and three ions at various times in the water above variously covered undisturbed cores (experiment 2). Solid line, naked mud (control); dotted line, sand-covered mud; broken line, polyethylene-covered mud. The points are the means for the five cores in each set, and the vertical lines beside them represent two standard errors above and below the means.

The results for nitrate-N did not differ markedly between the three sets of tubes. The averages ranged from 0.066 to 0.091 mg/liter on day 12 and they declined slowly to from 0.047 to 0.048 by day 55, presumably because of chemical reduction.

Figure 2 shows that the sand caused the oxygen concentration to decline more slowly than in the controls; both sand and polyethylene greatly reduced the rate of release of phosphate and iron and both impeded the release of nitrogen, the sand being the less effective.

Surprisingly, during this experiment, although it was done in the dark, Cyanophyta (*Oscillatoria* and some *Arthrospira*) grew on the polyethylene sheets from day 4 onwards, and similar growths appeared from day 11 onwards on the mud in two of the controls and from day 31 on the sand in two tubes.

EXPERIMENT 3

This was designed to investigate further the behaviour of disturbed mud, and in particular the release of iron and ionic nitrogen, which we had not previously studied.

Mud was collected by grab from Paradise Lake on July 26, 1967, and after thorough mixing 9 liters were placed in each of two glazed ceramic sinks, where they formed layers 4 cm deep. The sinks were 55.5 × 40.5 × 21.5 cm deep and their bottom outlets were fitted with clamped tubes that projected 1.5 cm above the mud surface. They were kept in the dark at 7 C.

After 1 day a brown ferric layer had developed on the mud surface, and settling of the mud had cleared a thin layer of supernatant water, which was removed by pipette. The mud in both tanks was covered with a sheet of polyethylene 0.01 cm thick, and lake water was carefully siphoned in to a depth of 17 cm (38.2 liters). In the control sink the polyethylene served merely to allow the water to be added without disturbing the mud, and when it floated up it was removed. In the other sink the sheet fitted closely to the walls and was weighted down with glass rods so that it could not move. The sinks were then covered with glass sheets that were sealed to their tops with silicone rubber cement. The sheets were 3 mm thick and each bore two spouts; the spout at one end was closed by a clamped tube, and that at the other by a stopcock. On day 3 the approximately 0.5-cm layer of air under the glass was replaced by nitrogen, and to each stopcock was attached a bag of this gas to prevent variations in atmospheric pressure from straining the glass. The silicone seals undoubtedly allowed the ingress of some oxygen but this did not seriously interfere with the experiment.

At the beginning conductivity of the lake water, which came from near the shore, was 152 μmho and *p*H was 8.25. Ionic concentrations (in milligrams/liter) were PO_4-P 0.010 (orthophosphate 0.004), Fe 0.03 (Fe^{++} 0.02), NO_3-N 0.062, NH_4-N 0.37, and SO_4 10.0. There was 4.20 mg/liter of oxygen. In the light of Rigler's (1968) findings it is possibly not meaningful to cite results for orthophosphate, and it is done here without prejudice.

The mud had a density of 1.035 g/ml and 7.8% dry matter, and contained (in milligrams) per gram of wet mud, total P 0.145, total Fe 2.4, and total N 1.34.

At intervals, as shown in Fig. 3, water was withdrawn through the bottom outlets for analysis. The nitrate-N had fallen to 0.05 mg/liter in the control and 0.03 over the polyethylene by day 11; thereafter it fell slowly until only traces remained on day 147. The polyethylene greatly impeded the release of iron, phosphate, and ammonium, and probably also of other ions that contributed to the conductivity (Fig. 3). It had little effect on *p*H, but it delayed

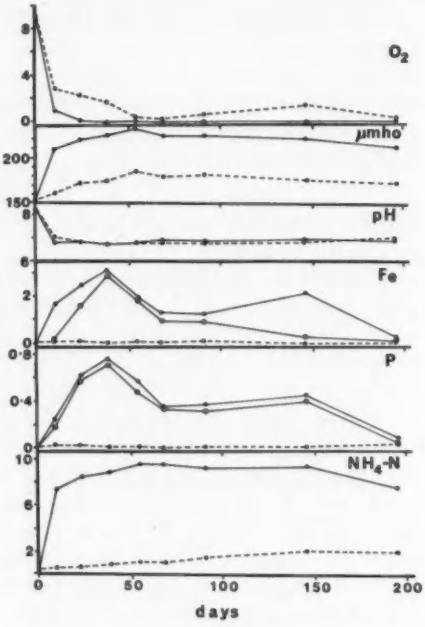


FIG. 3. Conductivity, *p*H, and concentrations of oxygen and three ions at various times in the water overlying disturbed mud (experiment 3). Values are shown in milligrams/liter except for conductivity (µmho) and *p*H. Solid lines, naked mud (control); broken lines, polyethylene-covered mud. The values for iron are shown as total iron (solid circles) and ferrous iron (open circles); those for phosphorus as total phosphate (solid circles) and orthophosphate (open circles).

deoxygenation of the water, probably by preventing release of organic matter, and this, coupled almost certainly with some leakage, resulted in small amounts of oxygen being always present, whereas the control rapidly became deoxygenated.

By day 39 the water samples from the control smelled of hydrogen sulphide, and a yellow scum began to form on the water surface. This material, which later proved to be ferric hydroxide with some bacteria, increased rapidly, and it was extensive by day 54. It almost certainly resulted from oxidation of ferrous ion by leaked-in air at the water surface, and was probably responsible for the decline in dissolved iron, and of phosphate by adsorption, after day 39. At the end of the experiment, on day 203, the water in the control sink was found to be very rich in bacteria and there were colonies on the walls. The appearance of the other sink had changed very little.

Twice during this experiment, on days 25 and 95, 50 ml of water was withdrawn from each sink and placed in a 125-ml flask. The flasks were then inoculated with a mixture of *Anabaena* and *Nostoc*, and were placed in a lighted place at room temperature. In both tests a single drop of Gaffion's minor elements was added to each flask, after 10 days in the first test and with the algae in the second; in both the growth of the algae was slow.

After 122 days and 111 days respectively the algae were collected on a 3-µ millepore filter, air-dried and weighed, with the following results in grams.

	25-day water	95-day water
Control	0.0523	0.0109
Polyethylene-covered	0.0004	0.0016

Clearly, therefore, although none of the water samples had been a good medium for growth of these algae under these experimental conditions, the control sink had provided the better medium.

MOVEMENT OF IONS THROUGH MUD

EXPERIMENT 4

This experiment was set up to check the indications in the literature that phosphate placed below the mud surface does not move into the overlying water.

On July 21, 1967, a core was taken in a type-3 tube from Paradise Lake so that the mud column was 42 cm deep beneath 7 cm of water, and it was stored in the dark at 7 C. After 39 days, by which time it was assumed that the water was deoxygenated (Fig. 2), about 0.4 ml of an aqueous solution of $NaH_2P^{32}O_4$ was slowly injected with a hypodermic syringe through the lateral slot into the centre of the core, 4 cm below the mud–water interface. Seven days before the injection the phosphate solution had contained 1 mc/0.5 ml.

At intervals during the next 64 days 0.5-ml samples of mud or water were withdrawn by syringe inserted laterally into the centre of the core, and their radioactivities were determined on a scintillator counter. The results (Table 1) were corrected for decay and against a blank sample of mud, but

TABLE 1. Counts per minute recorded for 0.5-ml samples withdrawn by syringe at various times from the water and from the mud at several levels below the interface of a core into which P^{32} had been injected at a depth of 4 cm (experiment 4). Counts corrected for decay back to day 7 and for blank of 100 cpm.

Depth below mud–water interface (*cm*)	Days after injection					
	8	17	24	36	51	64
Water	1,932	44,840	55,646	231,784	197,832	237,050
2	2,611,328	–	–	–	–	–
4	–	–	–	–	–	–
6	1,122,806	–	–	–	–	–
8	23,658	98,038	203,380	281,944	–	303,326
10	–	4,922	21,596	45,630	–	105,440
12	–	0	108	8,746	9,016	21,300
14	–	–	–	–	0	1,874

no correction was made for self-shielding by the mud particles because the syringe withdrew a rather more aqueous mixture than the mud itself. A correction based on the whole mud would have been erroneous, so the figures

in the table are only relative. They do, however, give some indication of the rate of movement of the P^{32}.

On day 66 the water was pipetted from above the core, and then successive layers of mud 1 cm thick were removed by pipette. The mud deeper than 13 cm was too firm to be effectively lifted by pipette so layers 14 and 15 were removed by spatula. The mud from each of the layers was thoroughly mixed in a beaker, as was also the water, and 0.5 ml was withdrawn with a syringe and assayed in the scintillator counter. In Fig. 4A the natural logarithms of

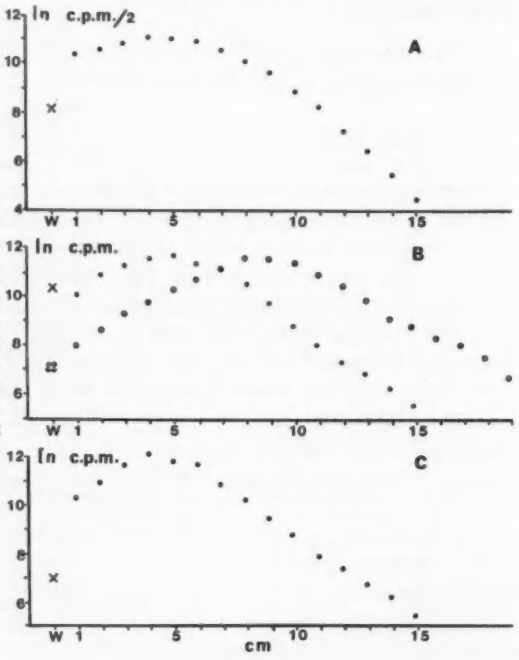


FIG. 4. Natural logarithms of the radioactivity of the mud at different depths 2 months after injection of P^{32} into undisturbed cores. A, core injected at 4 cm depth (experiment 4). B, open circles, core injected at 8 cm depth; solid points, core injected at 4 cm depth (experiment 5). C, sterilized core injected at 4 cm depth (experiment 5). W represents the overlying water and crosses the values obtained from it. Counts corrected against blank but not for decay and self-shielding.

the counts are plotted against depth, and then the slope of a line joining the points becomes inversely proportional to the diffusion coefficient of the P^{32}.

$$C = E\frac{dC}{dZ}$$

where C is the concentration (counts/½ min), E the diffusion coefficient of the P^{32}, and Z the depth. Then, assuming that E is not a function of Z, integrating and rearranging

$$\ln C = \frac{Z}{E} + K.$$

The graph shows a sharp change of slope on each side of the point of injection (4 cm) as would be expected, and the count for the water, which was 7 cm deep and probably subjected to turbulence and adsorption onto the Plexiglas, is not on the same general line as the points for the mud. This is, of course, to be expected, but the apparent change in slope at about 10 cm remains unexplained. It may have been caused by the alteration in the nature of the mud that, as already mentioned, occurs at about that depth (but see experiment 5 below), or it may have resulted from interference caused by our withdrawal of several syringe samples from about this depth during the experiment (Table 1).

EXPERIMENT 5

This was set up to determine whether there really was a change in the diffusion coefficient for P^{32} at about 10 cm depth, and if so to determine its cause, and to find out if the movement of phosphate was caused by bacteria or whether it was a purely physical phenomenon.

Three cores were obtained from Paradise Lake on September 19, 1968, in tubes of type 4, and were stored in the dark at 15 C. After 21 days, when deoxygenation of the water could be considered to be almost or quite complete, one core, which had 17 cm of overlying water and a colour change in the mud at a depth of 10 cm, was injected with formalin.

It had previously been determined, by making plate counts of bacteria grown on nutrient agar by the serial dilution method, that the upper few centimeters of the mud produced about 20–27 $\times$ 10^4 colonies/ml, and that this count was reduced to zero 2 days after the addition of sufficient formalin (40% formaldehyde) to raise the formaldehyde concentration to 0.5%. To sterilize the 720 ml of mud and the 475 ml of overlying water, the addition of 14 ml of formalin with the maximum of dispersion and the minimum of disturbance to the core was needed. This was done by slow injection of the solution with a hypodermic syringe through the rubber-filled slot. Five ml were injected into the water; 0.75 ml were injected into the mud at each of 10 points 1.5 cm distant from one another in such a way that 0.25 ml were distributed at three equidistant points in the horizontal plane; and 1.5 ml were similarly injected right at the bottom of the slot. The core was then left for a further 43 days at 15 C to allow the formaldehyde to disperse evenly.

Then, 63 days after collection, the temperature was lowered to 5 C and the next day all three cores were injected, as in experiment 4, with 1 ml of a 1 mc/ml solution of $NaH_2P^{32}O_4$. The sterilized core was injected 4 cm below the mud–water interface as was another core, which also had 17 cm of water and a colour change of the mud at 10 cm. The third core, which had 15 cm of water and a colour change in the mud at 12 cm, was injected at 8 cm below the interface.

The cores were left undisturbed for 66 days at 5 C, and were then dismantled, and the radioactivities of the various layers were determined as in experiment 4 (Fig. 4B and C).

In comparing these results with those in Fig. 4A it should be noted that only the patterns are comparable, not the absolute counts. This is because in experiment 4 the counts were per half-minute, only 0.4 ml of solution was injected, and it had longer to decay (66 + 7 days). The differences tend to cancel one another but there remain absolute differences of about 0.5–1.0 between comparable values of ln C in the two experiments.

Figure 4 shows that the change in slope at about 10 cm did not reappear in experiment 5, and that no change of slope accompanied the changes in colour of the mud. It is also clear that the formalin had no effect on the distribution of the phosphate, so the movement is not a biotic process.

Experiment 6

This experiment followed experiment 4 in the sequence of our work, and was performed to obtain statistically assessable data on the movement of phosphate through mud, and to see if there was a similar movement of the ammonium ion.

Fifteen cores were obtained in tubes of type 2 from Paradise Lake on August 25, 1967, and the interfaces were lowered so that they lay 1 cm below the bottom spout. At the same time large gelatinous colonies of *Nostoc* that occurred on the mud surface of some cores were removed with forceps, and lake water was added to fill the tubes. The tubes were placed in the dark at 7 C and, on day 14, 2 ml of a precooled solution of $(NH_4)_2HPO^4$ was slowly injected into the mud through the rubber-filled slots in 10 of the cores. It was injected into five cores 2 cm below the interface and into another five at 4 cm below the interface; the remaining five cores served as controls.

The 2 ml of solution injected into each core contained 0.266 g PO_4-P and 0.237 g NH_4-N and had been acidified to keep the ammonium in the ionic condition. This amount of salt spread evenly through the 89.6 ml occurring in a 4-cm layer of the core (2 cm up and down from the point of the injection) would about treble the amount of N in the mud (1.34 mg/g) and increase to about 21 times the amount of P (0.145 mg/g).

The cores were left for 70 days and then water samples were withdrawn from the bottom spouts for analysis, with the following mean results (in milligrams/liter ±1 SE):

	NH_4-N	PO_4-P
Control	8.92±0.59	0.59±0.089
Injection at 2 cm	163.2±12.01	39.1±2.01
Injection at 4 cm	167.0±6.77	30.3±1.87

Clearly both ions had moved up readily from both 2 and 4 cm down in the mud.

Experiment 7

Previous workers who have studied the movement of phosphate through mud, and have concluded that there is very little vertical movement, were

working with unproductive, mostly peaty, muds. As our results, in experiments 5, 6, and 7, were so much at variance with their findings we wondered if this was a peculiarity of calcareous eutrophic muds and not a general phenomenon. Experiment 7 was therefore a repetition of much of experiment 6, using an acid peaty mud.

Ten cores were obtained in type-2 tubes from Cranberry Bog on December 1, 1967. The water, which was about 1 m deep, had recently frozen over and the sediment was very soft and loose. The *p*H of both the mud and the water was 5.8, and the mud contained 1.21 mg/g wet weight of N and 0.016 mg/g of P, as determined from a sample taken from the top 10 cm.

As in experiment 6 the interfaces were lowered to 1 cm below the lowest spout, the water level was made up with water from the lake, and the tubes were stoppered and stored at 7 C in the dark.

Eight days later 2 ml of the same precooled solution of $(NH_4)_2HPO_4$ as was used in experiment 6 was injected slowly into five of the cores 4 cm below the interface, and the tubes were left undisturbed.

Seventy days after the injection water samples were withdrawn for analysis with the following mean results, (in milligrams/liter ±1 SE):

	NH_4-N	PO_4-P
Control	3.31±0.120	0.0772±0.0245
Injected at 4 cm	87.6±7.56	7.54±0.867

Once again it is clear that both ions had moved up readily through 4 cm of undisturbed sediment.

DISCUSSION

We conclude from our experiments that both a thin sheet of polyethylene and a layer of sand placed over the surface of mud below deoxygenating water greatly impede the release of phosphate, ammonium, and iron, and that although sand, for some unexplained reason, decreases the rate of loss of oxygen more than does polyethylene, it is a less effective barrier to ammonium (experiment 1). These findings apply to undisturbed mud surfaces as well as to mixed mud, and it seems that the prevention of the release of ions from the mud results in the water being a less suitable medium for algal growth.

We also conclude that the suggestions of earlier investigators that only a thin upper layer of the mud may be involved in the exchange of phosphate with the water are erroneous, possibly because their experiments were not long enough. Our study shows that, given time, both phosphate and ammonium move readily through anaerobic mud.

The hypothesis that the phosphate in our experiments may have moved only because it was introduced in already soluble form into deoxygenated mud, whereas had it been deposited from above when oxygen was present it might have been immobilized in organic compounds, appears to be untenable. Olsen (1958b, 1964) demonstrated that phosphate is exchanged between mud and

water both in the presence and in the absence of oxygen. Thus, even though the rate of exchange may be lower in aerobic conditions, some phosphate is freed at all times. Also, of course, the aerobic layer of the mud is always thin, so later deposits bury sedimented matter into a reducing zone, where, as Olsen showed, the rate of release is higher. Then, once released, it would move as in our experiments. Most of our cores were not sterile and presumably the normal microbial flora was present, yet there was no evidence of biotic immobilization of the introduced phosphate. Indeed the results from the one sterile core very closely resembled those from the nonsterile ones (Fig. 4), which indicates not only that anaerobic bacteria do not prevent movement of extra phosphate but that it moves by simple diffusion through mud. The same probably applies to ammonium and ferrous iron.

As phosphate and presumably also ammonium and ferrous iron move downwards as well as upwards, and as the concentrations of at least the important nutrients N and P are relatively very high in lake muds, a very large store of these elements must be built up in the sediment of a lake that becomes eutrophic because of pollution. This then remains as a continual supply for release into deoxygenated water for long periods after the addition of extra nutrients has ceased. It is probable therefore that to restore seriously altered lakes to a useful condition in a reasonable time it will be necessary to remove much of the sediment, or to lock the accumulated nutrients into it. We once again suggest that weighted polyethylene sheet, laid in overlapping strips to allow the escape of gases, may well provide a simple answer to this problem, at least for small and valued lakes. Presumably new sediment would collect very soon on the sheets and allow the reestablishment of the benthic fauna.

As far as we are aware there is no feature in our experiments that would require any but a straightforward interpretation of their results, and indeed the different experimental approaches gave, apparently unequivocally, similar results.

Possibly there is a future for polyethylene sheeting, or perhaps sand or some similar material, in applied limnology. It is conceivable, though, that as phosphate can move through mud it would, given long enough, ultimately move through sand. Plastic sheets, which seem to remain intact for long periods even in the soil, would therefore provide a better long-term solution to the rehabilitation of lakes that have been made eutrophic by man and have then ceased to receive pollution.

Finally, while we are on the subject of the possible use of plastic sheeting in the rehabilitation of lakes, we wonder if it may not have other uses. Would it not, for instance, be possible to use fairly thick-walled, but flexible, plastic tubes to carry effluents, or even small tributaries that contain undesirable quantities of nutrients, right through small lakes where high quality water was to be preserved? Similarly, could houses that now surround so many lakes, and destroy them by seepage from cesspits and similar devices, not drain their effluent via some suitable treatment pit into a flexible ring sewer laid deep in the water out of sight and below the frost line?

ACKNOWLEDGMENTS

The work was supported by a grant from the National Research Council of Canada for which we are grateful. We are also indebted to Dr W. E. Inniss of our department for assistance with our work with bacteria and radiophosphorus, and to Dr H. M. Hill of the Department of Civil Engineering for guidance in the interpretation of the results of our experiments on diffusion of P^{32} through mud.

REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION. 1965. Standard methods for the examination of water and wastewater. 12th ed. APHA, New York. 769 p.

GESSNER, F. 1939. Die Phosphorarmut der Gewässer und ihre Beziehung zum Kalkgehalt. Int. Rev. Ges. Hydrobiol. Hydrogr. 38: 202–211.

HAYES, F. R. 1955. The effect of bacteria on the exchange of radiophosphorus at the mud–water interface. Verh. Int. Ver. Theor. Angew. Limnol. 12: 111–116.
1964. The mud–water interface. Oceanogr. Mar. Biol. Annu. Rev. 2: 121–145.

HAYES, F. R., J. A. MCCARTER, M. L. CAMERON, AND D. A. LIVINGSTONE. 1952. On the kinetics of phosphorus exchange in lakes. J. Ecol. 40: 202–216.

HAYES, F. R., AND J. E. PHILLIPS. 1958. Lake water and sediment. IV. Radiophosphorus equilibrium with mud, plants, and bacteria under oxidized and reduced conditions. Limnol. Oceanogr. 3: 459–475.

HEPHER, B. 1958. On the dynamics of phosphorus added to fish ponds in Israel. Limnol. Oceanogr. 3: 84–100.
1966. Some aspects of the phosphorus cycle in fish ponds. Verh. Int. Ver. Theor. Angew. Limnol. 16: 1293–1297.

HOLDEN, A. V. 1961. The removal of dissolved phosphate from lake water by bottom deposits. Verh. Int. Ver. Theor. Angew. Limnol. 14: 247–251.

HUTCHINSON, G. E. 1957. A treatise on limnology. Wiley, New York. 1015 p.

HYNES, H. B. N., AND B. J. GREIB. 1967. Experiments on control of release of phosphate from lake muds. Proc. Conf. Great Lakes Res. 10: 357–362.

JUDAY, C., E. A. BIRGE, AND V. W. MELOCHE. 1941. Chemical analyses of the bottom deposits of Wisconsin lakes II. Trans. Wis. Acad. Sci. Arts Lett. 33: 99–114.

MACKENTHUN, K. M. 1968. The phosphorus problem. J. Amer. Water Works Ass. 60: 1047–1054.

MACKERETH, F. J. H. 1963. Some methods of water analysis for limnologists. Sci. Publ. Freshwater Biol. Ass. 21: 70 p.

MACPHERSON, L. B., N. R. SINCLAIR, AND F. R. HAYES. 1958. Lake water and sediment III. The effect of pH on the partition of inorganic phosphate between water and oxidized mud and its ash. Limnol. Oceanogr. 3: 318–326.

OHLE, W. 1953. Der Vorgang rasenter Seenalterung in Holstein. Naturwissenschaften 40: 153–162.

OLSEN, S. 1958a. Fosfatbalancen mellem bund og vand i Furesø in Berg K. et al. Furesøundersøgelser 1950–54. Folia Limnol. Scand. 10: 39–96.
1958b. Phosphate adsorption and isotopic exchange in lake muds. Verh. Int. Ver. Theor. Angew. Limnol. 13: 915–922.
1964. Phosphate equilibrium between reduced sediments and water. Laboratory experiments with radioactive phosphorus. Verh. Int. Ver. Theor. Angew. Limnol. 15: 333–341.

POMEROY, L. R., E. E. SMITH, AND C. M. GRANT. 1965. The exchange of phosphate between estuarine water and sediments. Limnol. Oceanogr. 10: 167–172.

PROFT, G. 1967. Beiträge zur Sedimentchemie von Binnengewässern. I. Die Verteilung des Phosphors in Sedimenten von Seen und Talsperren. Limnologica 5: 397–404.

RIGLER, F. H. 1956. A tracer study of the phosphorus cycle in lake water. Ecology 37: 550–562.

1964. The phosphorus fractions and the turnover time of inorganic phosphorus in different types of lakes. Limnol. Oceanogr. 9: 511–518.

1968. Further observations inconsistent with the hypothesis that the molybdenum blue method measures orthophosphate in lake water. Limnol. Oceanogr. 13: 7–13.

SULLIVAN, W. T. 1967. Chemical composition of the mud–water interface zone, with the description of an interface sampling device. Proc. Conf. Great Lakes Res. 10: 390–403.

ZICKER, E. L., K. C. BERGER, AND A. D. HASLER. 1956. Phosphorus release from bog lake muds. Limnol. Oceanogr. 1: 296–303.

Diel Vertical Migration of *Pandalus borealis* in Kachemak Bay, Alaska

LOUIS BARR

Bureau of Commercial Fisheries
Biological Laboratory, Auke Bay, Alaska 99821, USA

BARR, L. 1970. Diel vertical migration of *Pandalus borealis* in Kachemak Bay, Alaska. J. Fish. Res. Bd. Canada 27: 669–676.

Catches of *Pandalus borealis* by surface-to-bottom pot fishing in Kachemak Bay, Alaska, showed that this species was distributed in large numbers throughout the water column at night and suggested a diel vertical migration. The shrimp apparently left the vicinity of the bottom about dusk and returned about dawn; the apparent length of time they were off bottom was directly related to the length of the night. Bottom trawling clearly showed lower availability of *P. borealis* on bottom at night than during the day. Catches of shrimp by pot fishing and trawling indicated that small *P. borealis* have a greater tendency to migrate vertically than the larger individuals.

Received June 2, 1969

INTRODUCTION

THOUGH PINK SHRIMP (*Pandalus borealis*) are usually captured by trawls fished on the bottom, some evidence has been given that this pandalid undergoes a diel vertical migration. Midwater captures were reported by Horsted and Smidt (1956) during sampling at night with a stramin net, by Aron (1959) and Rathjen and Fahlen (1962) in exploratory midwater trawling, and by Nansen (1924) with bag nets. Blacker (1957) reported occasional captures at depths as shallow as 30 fath where the bottom was at 120 fath. Several authors (Wollebaek, 1904; Hjort and Ruud, 1938; Horsted and Smidt, 1956) noted that shrimp fishermen generally believe that *P. borealis* rises from the bottom at night, as catches on the bottom are poorer at night than during the day. However, no research specifically on vertical distribution of *P. borealis* has been reported in the literature.

As part of a research program on Alaska pandalid shrimps by the U.S. Bureau of Commercial Fisheries, I investigated the vertical distribution of *P. borealis* and the changes in this distribution in daylight and darkness. The investigation was carried out at a Bureau research station in Kachemak Bay, which is located at the southwest tip of the Kenai Peninsula and opens into lower Cook Inlet. In this paper I describe the diel vertical distribution of *P. borealis* in Kachemak Bay at different times of the year and discuss the evidence of diel vertical migrations of this species.

RATES OF CATCH OF SHRIMP AT VARIOUS DEPTHS IN DAYLIGHT AND DARKNESS

The diel vertical distributions of shrimp were determined by surface-to-bottom pot fishing (Barr and McBride, 1967) for 24-hr fishing periods. Sampling

Printed in Canada (J1496)

was done in August and September 1965, April and June 1966, and January 1967. In each period two strings of pots were fished about 150 m apart in about 90 m of water. The depth of the water varied throughout each sampling period because of tidal changes; the mean tidal range was 5.5 m. Each string had five pots: one on bottom, one just beneath the surface, and three spaced about 23 m apart along a vertically suspended line (Fig. 1). One baiting with chopped

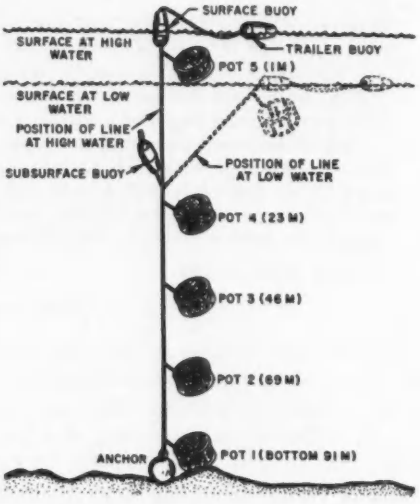


FIG. 1. Diagrammatic illustration (not to scale) of a surface-to-bottom string of pots set to fish for shrimp, Kachemak Bay, Alaska. (Modified from Barr and McBride, 1967.)

herring was used in each pot for the 24-hr sampling period. Both strings were set at noon and pulled at the end of each 3-hr interval through noon of the following day. At each lifting of the gear, the catch was removed and the pots reset in about the same location. Slight variations in location were unavoidable because the boat drifted during the 10 min needed to pull, empty, and reset the pots. The catch data from the two strings of pots are combined for all analyses.

The sampling periods are arranged in order of increasingly longer nights to show more clearly the relation of the vertical distribution of the shrimp to the length of the period of darkness. Daylight and darkness were delimited by the estimated times of sunrise and sunset at 59°N. These times were obtained by interpolation from the table given by the U.S. Coast and Geodetic Survey (1966) and rounded to the nearest quarter hour.

The catches of *P. borealis* throughout the water column were closely related to daylight and darkness during each of the five sampling periods (Fig. 2). Shrimp taken during daylight were usually caught in the pots set on bottom or at the first level above bottom. Only during the June sampling period were shrimp caught as high as the middle level before sunset. After sunset and during all of the dark intervals, shrimp were caught throughout all, or most,

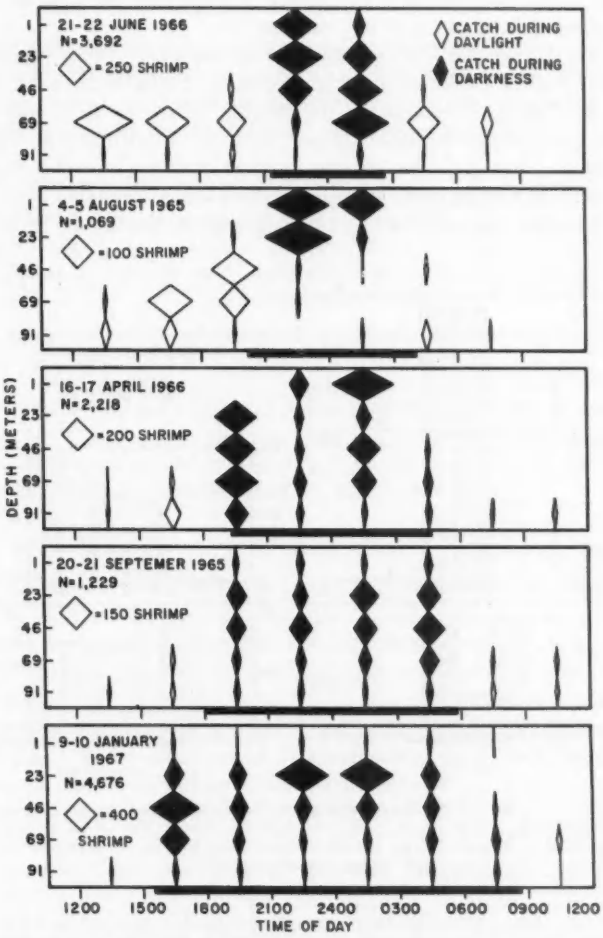


FIG. 2. Total catches of *Pandalus borealis*, by time and depth, taken by surface-to-bottom fishing of two strings of pots in Kachemak Bay, Alaska. Time between sunset and sunrise is indicated by heavy black line along horizontal axis. Sampling periods are arranged in order of increasing length of the night.

of the water column. The upward shift of catches was even apparent in the 6:00 PM to 9:00 PM interval in August, when sunset occurred less than an hour before the pots were pulled. Catches at the upper two levels in the water column decreased or ceased during the interval including sunrise or the next later interval. Thus, although the total catches fluctuated strongly between fishing intervals, the catches at each depth show a rise of the shrimp into the upper

part of the water column at night and a subsequent settling of the shrimp toward bottom at or soon after sunrise.

The total catches of shrimp (all depths combined) were higher for each 3-hr interval during darkness than in those by daylight for each of the five 24-hr periods (Fig. 3). The ratio of the average catch per 3-hr interval during darkness to that during daylight ranged from 3.5:1 (August) to 70.4:1 (January) (Table 1). The difference between catches by daylight and in darkness was least pronounced during the long-day, short-night periods, and was most pronounced during the short-day, long-night periods.

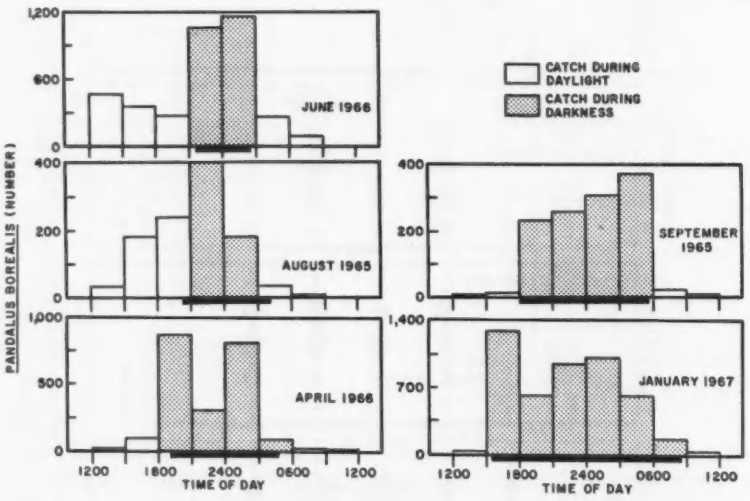


FIG. 3. Total catches (all depths combined) of *Pandalus borealis* by surface-to-bottom fishing of two strings of pots for each 3-hr interval during five 24-hr fishing periods in Kachemak Bay, Alaska. Time between sunset and sunrise is indicated by heavy line along horizontal axis.

TABLE 1. Average total pot catches of *P. borealis* (all depths combined) per 3-hr fishing interval in daylight and darkness and ratios of the catches in daylight to those in darkness for five 24-hr periods in Kachemak Bay, Alaska, 1965–67.

Fishing period	Avg no. shrimp caught per 3-hr interval		Ratio of catches Daylight: darkness
	Daylight	Darkness	
June 1966	243	1116	1:4.5
Aug. 1965	82	289	1:3.5
Apr. 1966	32	523	1:16.3
Sept. 1965	14	293	1:20.9
Jan. 1967	11	775	1:70.4

Relation of Size of Shrimp to Depth of Capture

The average weights of shrimp captured at various depths were examined for evidence of size segregation by depth. Differences in the average weight of *P. borealis* captured in pots at different depths were apparent, especially between shrimp caught on the bottom and in midwater (Fig. 4). Large and

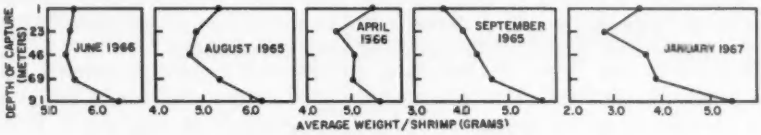


FIG. 4. Relation of average weight per shrimp to depth of capture in surface-to-bottom pot fishing in Kachemak Bay, Alaska.

small *P. borealis* were taken at every level, but the proportion of small shrimp was usually much greater in catches from above bottom than in those from the bottom. As a result, the average weight of shrimp captured on bottom was always greater than for those taken at any other level.

Relation of Distribution of Shrimp to Temperature and Salinity

Water temperature and salinity in the sampling area were measured with an inductive salinometer at three depths (surface, 43 m, and 86 m) during each period of pot sampling. *Pandalus borealis* were captured in pots throughout the water column regardless of the temperatures and salinities. They were taken at temperatures ranging from 1.3 C (April 1966) to 9.4 C (September 1965), including temperature gradients between surface and bottom as great as 3.3 degrees C (June 1966); and at salinities of 23.4‰ (January 1967) to 30.2‰ (August 1965), including salinity gradients between surface and bottom as great as 6.5 parts per thousand (September 1965).

Abundance and Size of Shrimp on the Bottom in Daylight and Darkness

A series of trawl hauls was made on the bottom in September 1966 to gather data on abundance and size of *P. borealis* on the bottom during daylight and darkness. A 3-m otter trawl with 6.4-mm mesh (square measure) in the codend was used for sampling. Four 10-min hauls were completed in daylight between 11:00 AM and 1:00 PM and four in darkness between the succeeding 11:00 PM and 1:00 AM. Abundance was measured by the total weight of *P. borealis* in each catch, and size was measured by the average weight of individual shrimp. The trawling was in a part of Kachemak Bay that has an extensive area of uniformly smooth bottom in about 73 m of water, about 16 km from the pot-fishing site (which is an untrawlable area).

The trawl catches showed that fewer shrimp were on bottom at night than during the day and that the shrimp on bottom at night had a larger average

size than those on bottom during the day. The average weight of the catch during darkness (2.5 kg) was only 29% of the daylight catch (8.7 kg), but the average weight of individual shrimp in the night catches (5.2 g) was 140% of those in the daylight catches (3.7 g). Although the ranges of average weight of individuals in the catches from daylight (3.3–4.0 g) and darkness (4.8–5.5 g) did not overlap, all sizes were represented in every catch. The increase in average size of shrimp during darkness was caused by a change in the proportions of large and small *P. borealis* in the catches — proportionally fewer small shrimp were captured in darkness than in daylight.

DISCUSSION

Evidence of Vertical Migrations

The results of this study provide strong evidence of diel vertical migrations in *P. borealis*. The catches of shrimp in surface-to-bottom strings of pots suggest that many *P. borealis* leave the vicinity of the bottom in the late afternoon or evening and distribute themselves through the water column within a few hours after sunset. The shrimp then apparently remain distributed through the water column during the night and return to the vicinity of the bottom about dawn.

The catches of shrimp in day and night bottom trawls provide further evidence of vertical migration by clearly showing a large reduction in the total abundance of shrimp on bottom at night compared with the abundance on bottom during the day.

The interpretation of the results of each of the sampling methods used in this study must be subjected to certain qualifications. In the pot fishing, catches of shrimp in the pots prove the presence of shrimp in the vicinity of the pot, but the lack of any catch of shrimp does not unquestionably prove the absence of shrimp in the vicinity. Shrimp may possibly be present throughout the water column at all times but may be vulnerable to capture in the baited pots only at night because, if they feed mostly at night, only then will they respond to the bait. Thus, there could be an apparent vertical migration, when actually a part of the population of shrimp is permanently pelagic but only vulnerable to capture by baited pots at night. Unlike the passively fishing pots, however, the trawl is an active collection device and is assumed to capture a fairly constant (but unknown) percentage of the shrimp in its path. The reduced availability of shrimp on bottom at night indicates that many of the shrimp on bottom during the day have moved upward into the water column at night.

The only apparent alternative explanation to the reduced catches of shrimp in nighttime bottom trawls would be that the shrimp burrow into the bottom at night, thus becoming less vulnerable to the trawl. This explanation appears unlikely. Personal observations of several species of pandalid shrimps, including *P. borealis*, during day and night diving in Alaskan waters have

shown that the pandalids observed had no tendency to burrow into the bottom at any time.

Although the evidence of vertical migrations of *P. borealis* is circumstantial, consideration of all aspects of the evidence leads to the definite conclusion that many *P. borealis* perform a diel vertical migration.

Possible Explanation of Vertical Migration

The most likely explanation for the performance of a diel vertical migration by *P. borealis* is that it is a feeding migration. Horsted and Smidt (1956), who found planktonic crustaceans in stomachs of *P. borealis*, theorized that vertical migrations of the shrimps might correspond to migrations of their food organisms. A preliminary study of the food and feeding behavior of *P. borealis* in Kachemak Bay showed that the shrimp are primarily zooplankton feeders and that most feeding is at night.[1] The shrimp for this food and feeding study, collected by surface-to-bottom pot fishing during daylight and darkness, showed that peak feeding activity at all depths of capture occurred during the early half of the night. The food item occurring most frequently in all the shrimp stomachs, regardless of depth of capture, was brachyuran crab larvae. No information was obtained on the vertical distribution of the crab larvae, but the usual planktonic existence of these organisms indicates that the shrimp fed most heavily at above-bottom levels of the water column.

Practical Significance of Vertical Migrations

Vertical migratory behavior in *P. borealis* is important in analysis of research fishing for this species. The results of any fishing effort could be greatly affected by the time of day when the fishing is conducted. Fishing with a bottom trawl at night might produce relatively few *P. borealis* in an area of great abundance simply because most of the shrimp were off bottom. In a population composed of *P. borealis* and other species with possibly different or no vertical migratory behavior, the species composition of bottom trawl catches might not represent the true population if the bottom trawling was conducted at a time of day when the *P. borealis* or other migratory species were not on bottom. Because bottom trawling is the principal sampling method now used for *P. borealis* and several other pandalid species in Alaska, the problems mentioned are real.

A diel vertical migration of *P. borealis* would have some interesting potential applications in commercial fishing. Foremost is the possibility of harvesting these shrimp by nighttime midwater trawling over untrawlable bottoms. Second is the possibility of taking "cleaner" catches of shrimp by midwater trawling, avoiding the large quantities of trash fish now taken in commercial shrimp catches in Alaska. A third potential application is in

[1]Based on a study of the food and feeding habits of *P. borealis* in Kachemak Bay, Alaska, during one 24-hr period in August 1967 (unpublished data).

nighttime bottom trawling, when total catches would be lower but average size of the harvested shrimp would be larger. This fishing would produce a higher quality catch while preserving the smaller shrimp, which are often discarded dead after being caught.

ACKNOWLEDGMENTS

I thank those who took part in the 30-hr sessions of work needed to gather the data for this report. R. McBride operated the RV *Sablefish* during all of the sampling. J. Moore, D. Segawa, and D. Richardson each participated in one or more of the sampling periods.

REFERENCES

ARON, W. 1959. Midwater trawling studies in the North Pacific. Limnol. Oceanogr. 4: 409–418.

BARR, L., AND R. MCBRIDE. 1967. Surface-to-bottom pot fishing for pandalid shrimp. U.S. Fish Wildlife Serv. Spec. Sci. Rep. Fish. 560: iv + 7 p.

BLACKER, R. W. 1957. Benthic animals as indicators of hydrographic conditions and climatic change in Svalbard waters. Fish. Invest., Min. Agr., Fish. Food (Great Britain), Ser. 1, Salmon Freshwater Fish. 10: 1–49 + append.

HJORT, J., AND J. T. RUUD. 1938. Deep-sea prawn fisheries and their problems. Hvalrådets. Skr. 17: 1–144.

HORSTED, S. A., AND E. SMIDT. 1956. The deep-sea prawn (*Pandalus borealis* Kr.) in Greenland waters. Medd. Dan. Fisk. Havunders. (N.S.) 1(11): 1–116.

NANSEN, F. 1924. Blant sel og bjørn. Minförste Ishavsferd. Jacob Dybwads Forlag, Christiania. 285 p. (Cited by Hjort and Ruud, 1938.)

RATHJEN, W. F., AND L. A. FAHLEN. 1962. Progress report on midwater trawling studies carried out off the New England coast in 1961 by M/V *Delaware*. Commer. Fish. Rev. 24(11): 1–11.

UNITED STATES COAST AND GEODETIC SURVEY. 1966. Tide tables, west coast of North and South America, including Hawaiian Islands, 1966. U.S. Govt. Printing Office, Washington, D.C. 224 p.

WOLLEBAEK, A. 1904. Raeker og raekefisket. Arsberetn. Vedkom. Norg. Fisk. 1903: 167–229.

Concentrations of Trace Elements in Great Lakes Fishes[1,2]

HENRY F. LUCAS, JR., AND DAVID N. EDGINGTON

Radiological Physics Division
Argonne National Laboratory, Argonne, Illinois 60439, USA

AND

PETER J. COLBY

Bureau of Commercial Fisheries
Great Lakes Fishery Laboratory, Ann Arbor, Michigan 48107, USA

LUCAS, H. F., JR., D. N. EDGINGTON, AND P. J. COLBY. 1970. Concentrations of trace elements in Great Lakes fishes. J. Fish. Res. Bd. Canada 27: 677–684.

The concentration of 15 trace elements was determined by activation analysis of samples of whole fish and fish livers from three of the Great Lakes: Michigan, Superior, and Erie. The average concentrations of 7 elements in 19 whole fish from 3 species were as follows: uranium, 3 ppb (parts per billion); thorium, 6 ppb; cobalt, 28 ppb; cadmium, 94 ppb; arsenic, 16 ppb; chromium, 1 ppm; and copper, 1.3 ppm. The average concentrations of 8 elements in 40 liver samples from 10 species of fish were as follows: uranium, ~2 ppb; thorium, $\leq$2 ppb; cobalt, 40 ppb; copper, 9 ppm; zinc, 30 ppm; bromine, 0.4 ppm; arsenic, 30 ppb; and cadmium, 0.4 ppm. Other elements observed in most of the samples were: antimony, 5–100 ppb; gold, 2–5 ppb; lanthanum, 1–20 ppb; rhenium, 0.5–5 ppb; rubidium, 0.06–4 ppm; and selenium, 0.1–2 ppb.

Trace element concentrations varied with species and lake. Uranium and thorium varied with species, but not for the same species from different lakes. The levels of copper, cobalt, zinc, and bromine varied little between species and lakes. The concentration of cadmium, arsenic, and chromium varied between species and with species between lakes.

Received November 24, 1969

INTRODUCTION

POLLUTION IN THE Great Lakes has received considerable attention in recent years. In particular, pollution from the discharge of large amounts of the major nutrients, nitrogen and phosphorus, has been given extensive publicity. In addition to nitrogen and phosphorus, however, large amounts of many other elements and compounds are regularly discharged into the lakes from industrial and agricultural sources. These trace contaminants may have effects on the ecosystem as great as or greater than those of the more common pollutants.

The complex role of trace elements in the biosphere is not fully understood, but one fact is known: trace elements in high concentrations are toxic to living organisms. At less than toxic concentrations, certain trace elements may be

[1]This work was performed under the auspices of the U.S. Atomic Energy Commission.
[2]Contribution No. 411, Great Lakes Fishery Laboratory, Bureau of Commercial Fisheries, Ann Arbor, Michigan 48107, USA.

Printed in Canada (J1651)

essential to physiological function of particular organisms or to the ecological system as a whole. Low concentrations of essential trace elements may suppress physiological action, leading to abnormal growth of an organism (Bowen, 1966). The difference in concentration between deficiency and toxicity is often small. Furthermore, the natural concentration is often very high so that a mere doubling of it may prove toxic to the more sensitive species (Bowen, 1966; Jones, 1964). The toxicity of an element appears to be directly correlated with the electronegativity or with the stability of the complexes of the metal ion, and is, therefore, probably due to the formation of the complexes in the proteins and enzymes at physiologically active sites (Doudoroff and Katz, 1953; Bowen, 1966).

In addition to information on the toxic properties of trace metals, extensive knowledge of their metabolism under natural conditions is required to evaluate the potential hazards of nuclear reactors that may be erected on the shores of the Great Lakes. Depending on the metabolic behavior of the individual elements and the species of aquatic life, trace metals are concentrated, maintained at a constant value, or are discriminated against at each of the various trophic levels (Gustafson et al., 1966). Because fish accumulate trace metals from their environment and can act as indicators of levels in the environment, they are excellent organisms for the study of some long-term changes in trace metals in the environment.

The relation between the pollution of rivers with heavy metals and the concomitant disappearance of fish was first studied in 1919 by K.E. Carpenter (Jones, 1964) who determined the toxicity limits of different heavy metals for a variety of species. The concentration of trace elements in fish from the Great Lakes has not been previously reported. It was the purpose of the present study to: (1) identify those trace elements detectable by neutron activation and other methods of analysis; (2) establish the means and ranges of concentration for several trace elements in different species of fish; and (3) identify the trace elements whose concentrations differ by species, lake, or collection site. A special effort was made to include elements known to be toxic, those released in industrial effluents, and those considered to be hazardous in the event of an accident at a nuclear power station.

METHODS

Fish were collected with trawls in Lake Michigan and Lake Erie and with trawls and gillnets in Lake Superior through experimental fishing by research vessels of the U.S. Bureau of Commercial Fisheries during 1968 and 1969. The depth, location, surface water temperature, and wind velocity and direction were recorded at each sampling station. The samples from Lake Michigan were obtained near the mouth of the Kalamazoo River off Saugatuck, Mich., and in deep water near Waukegan, Ill. The Lake Erie samples were from East Harbor near Sandusky, Ohio, and those from Lake Superior were collected off Basswood Island near Bayfield, Wis. All fish were weighed, measured (total length), and sexed, and scales were removed for age determination. Either whole fish (for three small species) or the livers (for fish of 10 other species, too large to analyze as whole fish) were taken. The livers were removed with a surgical steel scalpel and teflon-coated tweezers. The individual whole fish or livers were placed in separate polyethylene bags and quickly frozen. All samples were stored at −10 C until time of analysis.

All of the fish from which the livers were removed were 2 years old or older. Because most samples for each species were from the same age-group, we could not determine the influence of age on the concentration of trace elements in fish livers.

The liver samples were prepared for analysis by freeze-drying followed by low temperature oxidation with ionized oxygen.[3] The power for oxidation was 100 w; the sample temperature did not exceed about 100 C. Portions of the liver ash were placed in polyethylene capsules and were irradiated with appropriate standards at an integrated flux of about 5×10^{16} neutrons/cm^2 in Argonne National Laboratory's CP-5 or Juggernaut reactor. Because whole fish could not be completely ashed by this procedure, it was necessary to heat them in quartz crucibles for 16 hr at 600 C. Some of the more volatile elements (mercury) were partially lost by this treatment. Aliquot samples of approximately 1 g wet weight (0.015–0.025 g ash) whole fish or fish liver were analyzed.

After irradiation, the samples were dissolved in 10 ml of 9 M HCl to which several drops of 30% hydrogen peroxide were added to reduce chromium to its trivalent state, and approximately 0.1 g aluminum nitrate was added to complex any fluoride ion present. This solution was then heated for several minutes in a boiling water bath after which the anions forming strong chloride complexes were adsorbed at a flow rate of 0.5 ml/min on a Dowex 2-X8 column 0.6 cm in diameter and 35 cm long. Groups of elements, normally about five fractions each containing up to 10 elements (13 isotopes), were obtained by varying the composition of the eluent (Edgington, 1967). The amount of each nuclide was determined by counts obtained with a 4 × 2-inch NaI (T1) crystal. The gamma-ray spectra for each standard and each fraction were determined with an ND-120 multichannel pulse height analyzer. An automatic sample changer limited the counting interval to 100 min or 200,000 total counts, whichever occurred first. The data for each of the gamma spectra were accumulated on computer-compatible magnetic tape (Lucas and Edgington, 1969).

The gamma spectra from the standards and all fractions of the samples are very complex because of the many photopeaks from the numerous radioisotopes present. The amount of each radioisotope in each fraction was calculated by a rigorous, iterative, least squares method over the energy range of 0.06–3.2 Mev (Parr and Lucas, 1964). This method is very precise, and can be used for the identification of up to 20 isotopes. These results are then corrected for radioactive decay between time of irradiation and time of counting. The amount of each element is then computed by using the ratio of the decay-corrected counting rates in the sample to that from a known weight of standard. The minimum concentrations of elements detected by these techniques (Table 1) were similar to those calculated by Wing and Wahlgren (1965).

Because of a few operational problems with the chemical procedure or with the electronic instrumentation, not all samples were processed with the same sensitivity, accuracy, or precision. For example, some uranium values in Table 3 are given to the nearest 0.1 parts per billion (ppb) whereas others are shown to be equal to or less than 2 ppb. Furthermore, some samples had unusually high concentrations of copper or arsenic, which limited the sensitivity for uranium or other nuclides; these discrepancies were considered in the tabulation of the data.

The common and scientific names of the species of fish mentioned in this report are those approved by the American Fisheries Society (Bailey et al., 1960): alewife, *Alosa pseudoharengus*; lake herring, *Coregonus artedii*; lake whitefish, *Coregonus clupeaformis*; bloater, *Coregonus hoyi*; round whitefish, *Prosopium cylindraceum*; lake trout, *Salvelinus namaycush*; American smelt, *Osmerus mordax*; goldfish, *Carassius auratus*; spottail shiner, *Notropis hudsonius*; trout-perch, *Percopsis omiscomaycus*; white bass, *Roccus chrysops*; yellow perch, *Perca flavescens*; walleye, *Stizostedion vitreum vitreum*.

CONCENTRATIONS IN WHOLE FISH

Whole fish used for trace element analyses were alewives, spottail shiners, and trout-perch. The analyses included food in the intestines. Because all

[3]Tracerlab Low Temperature Asher, Model LTA 600 Large Sample Assembly.

TABLE 1. Experimental detection limits by neutron activation analysis — irradiated for 7 hr at a flux of 2×10^{12} neutrons/cm^2 per sec.

Element	Nuclide measured	Half-life	Sensitivity (μg)
Antimony	^{122}Sb	2.8 days	2.8×10^{-4}
Arsenic	^{76}As	26 hr	2.5×10^{-5}
Barium	^{131}Ba	11.5 days	5.4×10^{-4}
Bromine	^{82}Br	36 hr	2.0×10^{-4}
Cadmium	^{115}Cd	53 hr	3.4×10^{-3}
Calcium	^{47}Sc	3.4 days	2.5×10^{0}
Cesium	^{134}Cs	2.07 yr	1.1×10^{-3}
Cobalt	^{60}Co	5.24 yr	7.5×10^{-3}
Chromium	^{51}Cr	28 days	8.4×10^{-2}
Copper	^{64}Cu	12.8 hr	1.3×10^{-4}
Gold	^{198}Au	65 hr	5.0×10^{-5}
Iron	^{59}Fe	45 days	10
Lanthanum	^{140}La	40 hr	5.4×10^{-5}
Mercury	^{203}Hg	47.2 days	3.0×10^{-2}
Molybdenum	^{99}Mo	66 hr	1.0×10^{-1}
Phosphorus	^{32}P	14.2 days	1.0×10^{-3}
Rhenium	^{186}Re	89 hr	5.4×10^{-4}
Rhenium	^{188}Re	17 hr	1.2×10^{-4}
Rubidium	^{84}Rb	18.7 days	5.9×10^{-2}
Scandium	^{46}Sc	83.9 "	5.6×10^{-4}
Selenium	^{75}Se	120 "	4.3×10^{-2}
Sodium	^{24}Na	15.0 hr	2.4×10^{-5}
Silver	^{110m}Ag	262 days	6.0×10^{-2}
Thorium	^{233}Pa	27 "	2.0×10^{-3}
Uranium	^{239}Np	2.35 "	1.0×10^{-4}
Zinc	^{65}Zn	250 "	4.6×10^{-1}
Zinc	^{69}Zn	13.8 hr	1.2×10^{-2}

the alewives were small (15–20 mm long), about 20 were combined for each analytic sample; spottail shiners (55–65 mm long) and trout-perch (60–95 mm long) were analyzed individually.

URANIUM AND THORIUM[4]

The levels of uranium and thorium varied among the three species of fish; concentrations of both elements were highest in spottail shiners, followed by alewife and trout-perch (Table 2). Values for uranium and thorium were higher in spottail shiners from Lake Michigan than in those from Lake Erie, but in trout-perch the amounts varied only slightly between lakes Michigan and Superior.

Analyses for thorium and uranium were also made of two samples of zooplankton[4] from Lake Michigan. The values of 36 ppb for thorium and 18

[4]Samples kindly supplied by Prof J. W. Winchester, Department of Meteorology and Oceanography, University of Michigan, Ann Arbor, Mich. 48104, USA.

TABLE 2. Average concentrations of trace elements in whole fish (x/g tissue).[a]

Species and lake	No. analyses	Uranium (*ppb*)[b]	Thorium (*ppb*)	Cobalt (*ppb*)	Copper (*ppm*)[b]	Arsenic (*ppb*)	Cadmium (*ppb*)	Chromium (*ppm*)
Alewife								
Michigan	2	2.6±1.2	6.0±1.2	29±14	0.85±0.05	23[c]	62±15	1.1±0.5
Spottail shiner								
Michigan	2	6.8±4.0	12±4	42±15	0.80±0.15	3[c]	–[d]	0.9±0.5
Erie	3	4.8±1.5	7[c]	25±4	1.2±0.2	3.5±3.0	100±30	10[c]
Trout-perch								
Michigan	8	0.7±0.1	3.2±0.6	24±2	2.7±0.6	43±12	76±8	1.6±0.2
Superior	4	0.9±0.1	1.7[c]	22±5	0.8±0.3	7±2	140±60	≤3
Grand avg	–	3	6	28	1.3	16	94	1

[a]Errors are either average deviations of the mean or standard errors from counting statistics, whichever was greater.
[b]ppb = parts per billion, ppm = parts per million.
[c]Estimate based on one determination.
[d]Not determined.

ppb for uranium were considerably higher than in fish. Possibly these two elements are discriminated against with increase of trophic level.

COBALT AND COPPER

The concentrations of cobalt and copper varied little among species or between lakes (an exception was the relatively high level (2.7 ppm) of copper in trout-perch from Lake Michigan). Since cobalt and copper are essential trace elements, their concentrations may be under physiological control (Bowen, 1966).

CADMIUM, ARSENIC, AND CHROMIUM

The concentrations of these trace elements varied according to species and lake. The values also varied among individual fish of the same species and, in Lake Michigan, according to collection site. This variability may indicate variations in habitat or be caused by chemical pollution. The mean concentration of arsenic in trout-perch appeared to be significantly higher in Lake Michigan (43 ppb) than in Lake Superior (7 ppb).

CONCENTRATIONS IN FISH LIVER

Concentrations of eight trace elements in liver samples from 10 species are given in Table 3.

URANIUM AND THORIUM

The mean concentration of uranium in fish livers (Table 3) ranged from 0.5 to 4.8 ppb and was similar to that in whole fish (Table 2). The uranium concentration varied with species; bloaters and round whitefish had higher

TABLE 3. Average concentrations of trace elements in livers of 10 species of fish (x/g tissue).[a]

Species and lake	No. analyses	Uranium (*ppb*)[b]	Thorium (*ppb*)	Cobalt (*ppb*)	Copper (*ppm*)[b]	Zinc (*ppm*)	Bromine (*ppm*)	Arsenic (*ppb*)	Cadmium (*ppm*)
Lake herring									
Superior	5	≤2	≤3	20[c]	12±6	38±15	0.2±0.1	69±30	1.6[c]
Lake whitefish									
Superior	4	≤0.5	4±1	≤10	2.4±0.1	23±1	0.1±0.1	6.4±0.7	0.3±0.1
Michigan	2	0.6±0.2	≤3	36±5	8.5±2.5	–[d]	–[d]	21±3	0.09±0.01
Bloater									
Superior	3	4.8±2.3	≤4	12±1	2.4±0.8	44±28	>0.007	63±17	0.7±0.4
Michigan	2	2.2±0.8	2.1±0.7	40±6	7.4±2.5	–[d]	–[d]	12[c]	0.4±0.1
Round whitefish									
Superior	6	4.8±3.4	≤0.5	47±8	3.8±2.0	11±5	0.5±0.3	5.6±3.4	0.4±0.1
Lake trout									
Superior	2	≤2	≤4	31±3	28±8	48±12	0.5±0.2	80±10	≤3
Michigan	2	≤2	≤4	36±4	21±10	–[d]	–[d]	18±3	0.06±0.02
Smelt									
Michigan	2	≤2	2[c]	13±4	1.5±0.1	–[d]	–[d]	≤20	0.07±0.01
Goldfish									
Erie	3	0.5±0.2	53[c]	–[d]	14±1	36±2	0.3±0.2	6[c]	1.4±1.1
White bass									
Erie	2	≤2	≤3	43±2	≤4	28±5	0.8±0.4	98[c]	0.2[c]
Yellow perch									
Erie	4	0.9±0.3	≤3	120±60	3±2	–[d]	–[d]	7±2	0.54±0.06
Walleye									
Erie	3	≤1	0.8[c]	45±25	≤4	24±3	0.8±0.5	98[c]	0.2[c]
Grand avg	–	~2	≤2	40	9	30	0.4	30	0.4

[a,b,c,d]As in Table 2.

concentrations than goldfish, yellow perch, or lake whitefish. Bloaters from Lake Superior contained twice as much uranium as bloaters from Lake Michigan. The estimation of values for uranium and thorium in smelt, white bass, walleye, lake herring, and lake trout was affected by high concentrations of arsenic or copper or both.

Cobalt, Copper, Zinc, and Bromine

These elements are essential for bacteria, plants, mammals, and presumably for fish (Bowen, 1966). Essential trace elements are usually characterized by relatively small variations within a species.

The mean concentration of cobalt in fish livers of different species ranged from 10 or fewer to 120 ppb. The average deviation in the concentration of cobalt within a species (except for yellow perch and walleye) was limited by counting statistics and not by variations among samples. The lower concentrations of cobalt in bloaters and lake whitefish from Lake Superior than in those from Lake Michigan may indicate that cobalt is not under homeostatic control. In contrast, cobalt concentrations in lake trout from Lake Michigan and Lake Superior were nearly the same. Although the lake trout were planted fish, the very great change in size should have eliminated any direct affect of the hatchery environment.

The concentration of copper ranged from 1.5 to 28 ppm and was similar to that in marine fish (2–15 ppm, see Vinogradov, 1953). The concentration of copper in individual samples varied more than for cobalt. Excess copper, known to be stored in mammalian livers, may be stored in fish livers as well (Schroeder, 1956). The concentrations of copper in lake whitefish and bloaters from Lake Superior were less than those for the same species from Lake Michigan but the concentrations in lake trout from the two lakes were similar.

Zinc and bromine concentrations ranged from 11 to 48 ppm and 0.007 or less to 0.8 ppm, respectively. The range for zinc is similar to the range (33–73 ppm) reported for marine samples (Vinogradov, 1953), and to the value (20 ppm) reported for freshwater fish (Chapman et al., 1968). The data were insufficient to measure differences in the same species between lakes.

Arsenic and Cadmium

The mean concentration of arsenic excluding single samples ranged from 5.6 to 80 ppb and differed according to species in the same lake and within the same species in different lakes. Arsenic is localized in the fat fraction of the liver and other organs and has been shown to vary between samples of the same species from different rivers (Vinogradov, 1953). The concentrations found in our study, however, are 10–1000 times lower than those in marine samples (Vinogradov, 1953).

The mean concentration of cadmium ranged from 0.06 to 1.4 ppm. Concentrations were lowest in smelt, whitefish, and lake trout in Lake Michigan. The highest "precisely measured" concentration of cadmium was in Lake

Erie goldfish. Cadmium concentrations were higher in lake whitefish, and possibly in bloaters and lake trout, from Lake Superior than from Lake Michigan.

OTHER TRACE ELEMENTS

In addition to the trace elements reported, many others were observed in most of the samples of whole fish and fish livers and could have been determined. The concentrations of these elements were estimated from known cross sections, abundance, and neutron flux obtained from appropriate standards. The following elements and estimated ranges of concentrations were found: antimony, 5–100 ppb; gold, 2–5 ppb; lanthanum, 1–20 ppb; rhenium, 0.5–5 ppb; rubidium, 0.06–4 ppm; selenium, 0.1–2 ppb. In all samples, the following elements were occasionally observed but were equal to or less than the indicated concentrations: barium, 0.2 ppm; cesium, 3 ppb; silver, 0.001 ppb; scandium, 2 ppb; and mercury, 10 ppb.

ACKNOWLEDGMENTS

We thank M. Bailey, E. Brown, H.D. Van Meter, and their associates in the U.S. Bureau of Commercial Fisheries for help in collecting and identifying the fish, and S. O'Donnell, P. Koehler, M. Thommes, and F. Markun of the Argonne National Laboratory for their cooperation and assistance.

REFERENCES

BAILEY, R. M., E. A. LACHNER, C. C. LINDSEY, C. R. ROBINS, P. M. ROEDEL, W. B. SCOTT, AND L. P. WOODS [ed.]. 1960. A list of common and scientific names of fishes from the United States and Canada. Amer. Fish. Soc. Spec. Publ. 2: 102 p.

BOWEN, H. J. M. 1966. Trace elements in biochemistry. Academic Press, New York, N.Y. 241 p.

CHAPMAN, W. H., H. L. FISHERY, AND W. M. PRATT. 1968. Concentration factors of chemical elements in edible aquatic organisms. Lawrence Radiat. Lab. Rep. UCRL-50564. 50 p.

DOUDOROFF, P., AND M. KATZ. 1953. Critical review of literature on the toxicity of industrial wastes and their components to fish. II. The metals as salt. Sewage Ind. Wastes 25: 802–839.

EDGINGTON, D. N. 1967. The estimation of thorium and uranium at the submicrogram level in bone by neutron activation. Int. J. Appl. Radiat. Isotop. 18: 11.

GUSTAFSON, P. F., S. S. BRAR, AND S. E. MUNIAK. 1966. Caesium-137 in edible freshwater fish. Nature 211: 843–844.

JONES, J. R. E. 1964. Fish and river pollution. Butterworth and Co. (Publishers) Ltd., London, England. 203 p.

LUCAS, H. F., AND D. N. EDGINGTON. 1969. Computer analysis of gamma ray spectra: validity of the results, p. 207–214. *In* J. R. DeVoe [ed.] Modern trends in activation analysis. Nat. Bur. Stand. Spec. Publ. No. 312, Vol. 2.

PARR, R. M., AND H. F. LUCAS. 1964. A rigorous least squares analysis of complex gamma ray spectra with partial compensation for instrumental instability. Inst. Elec. Electron. Eng. Trans. Nucl. Sci. NS-11(3): 349–357.

SCHROEDER, H. A. 1956. Trace metals and chronic diseases, p. 259–303. *In* W. Dock and I. Snapper [ed.] Advances in internal medicine. Vol. 8. The Yearbook Publishers, Inc.

VINOGRADOV, A. P. 1953. The elementary chemical composition of marine organisms. Sears Found. Mar. Res., New Haven, Connecticut. 647 p.

WING, J., AND M. A. WAHLGREN. 1965. Detection sensitivities in thermal-neutron activation. Argonne Nat. Lab. Rep. ANL-6953. 107 p.

Distribution, Growth, and Seasonal Abundance of *Hyalella azteca* (Amphipoda) in Relation to Sediment Microflora[1]

BARRY T. HARGRAVE[2]

Department of Zoology
University of British Columbia, Vancouver 8, B.C.

HARGRAVE, B. T. 1970. Distribution, growth, and seasonal abundance of *Hyalella azteca* (Amphipoda) in relation to sediment microflora. J. Fish. Res. Bd. Canada 27: 685–699.

Growth, density, and body size of the deposit-feeding amphipod *Hyalella azteca*, and its food, epibenthic algae, and sediment microflora, were greatest in shallow-water areas of Marion Lake. The vertical distribution of *Hyalella* was limited to the upper 2 cm of sediment cores. Highest concentrations of sedimentary chlorophyll and lowest concentrations of nondigestible ligninlike material also occurred at the sediment surface.

In laboratory substrate-choice experiments, *Hyalella* differentiated between sediments with different concentrations of microorganisms, and growth depended upon the quantity of microflora in the diet. In Marion Lake, increased growth of *Hyalella* during June was independent of temperature and closely correlated with increased rates of epibenthic primary production.

Egg production, related to body size in a nonlinear manner, began during May as growth rates increased. As a combined result of egg production and juvenile survival, the maximum density of *Hyalella* in Marion Lake was reached in August.

Received April 24, 1969

INTRODUCTION

COMPARISON OF ECOLOGICAL CONDITIONS within areas of high and low density of animal species may suggest factors that influence their distribution. The present study considers the distribution of a freshwater benthic amphipod, *Hyalella azteca* (Saussure), from such a point of view.

Previous studies in Marion Lake have shown that *Hyalella* is most abundant in shallow-water sediment (less than 2 m) and almost absent from the deeper parts of the lake (5 m) (Hamilton, MS, 1965; Mathias, MS, 1967). This depth distribution corresponds directly to the attenuation of light and decrease in epibenthic algal production with depth in Marion Lake (Hargrave, 1969). Laboratory experiments have also indicated that *Hyalella* digests bacteria and algae from ingested sediment particles (Hargrave, 1970). Thus, if this food source is important in determining amphipod distribution, some relation between epibenthic primary production and numbers of *Hyalella* would be expected. This study compares the horizontal and vertical distribution, daily growth rate, and seasonal abundance of *Hyalella* with sediment microflora production and indices of digestible sediment organic matter.

[1]Canadian Contribution to the International Biological Programme No. CCIBP-52.
[2]Present address: Freshwater Laboratory, Hillerød, Denmark.

Printed in Canada (J1458)

MATERIALS AND METHODS

All materials were taken from sediments in Marion Lake, B.C., previously described by Efford (1967). Undisturbed dredge samples were taken at least once per month during 1968 by methods described by Hargrave (1969). The soft ooze sediments (gyttja) from various depths of the lake were either sieved (mean mesh size 0.25 mm) or sampled by glass cylinders (12 × 5 cm) (Hargrave, 1969). This allowed both total numbers per unit area and the vertical distribution of *Hyalella* within sediment cores from different areas to be compared with different measures of sediment organic matter.

Hyalella was separated from sediment sections by sieving, so that amphipods became trapped in the surface film of water over the sediment. Movement of animals trapped in the surface film allowed even the smallest instar stages to be collected. The densities of amphipods enumerated in this manner compared well with samples taken by the sugar flotation technique (Anderson, 1959) as used by Mathias (MS, 1967). Amphipods were enumerated, eggs were counted when present, and all animals were dried at 50 C for 12 hr before weight determinations were made with a Cahn electrobalance (±1 μg) in an external weighing chamber held at 50 C. This drying temperature prevented loss of volatile organic substances, as recommended by Lovegrove (1966).

Sectioning of sediment cores was done immediately after the core was taken by pushing the lower end of the core with a plunger and slicing consecutive 1-cm sections from the top with a sharp edge. When amphipods had been separated from each section, sedimentary chlorophyll and acid-insoluble matter were determined (Hargrave, 1969, 1970).

The importance of microflora on sediment as food for *Hyalella* was examined by measuring the changes in dissolved oxygen concentration over undisturbed sediment cores, which provides an index of benthic algal production. The technique is described by Hargrave (1969).

The vertical distribution of oxygen and *p*H in undisturbed sediment cores was measured by inserting a Beckman oxygen probe and *p*H electrode into the sediment immediately after sampling. Care was taken not to disturb stratification within sediment cores.

Substrate-choice experiments were used to determine the effect of sediment microflora on *Hyalella* distribution. Substrate-choice experiments have been used to demonstrate the ability of benthic invertebrates to select substrates of a certain grain size (Wieser, 1956) or particles with certain microorganisms on their surfaces (Wilson, 1955; Meadows, 1964; Marzolf, 1965; Gray, 1967).

During each of the four substrate-choice experiments, *Hyalella* was offered surface sediment treated to alter the microflora content. Diatoms (*Navicula* sp.) were cultured from Marion Lake sediment. A slurry of cells and sediment was filtered by vacuum to increase cell numbers in the sediment. Streptomycin-(SO_4) and neomycin (50 mg/liter) were used to inhibit bacterial growth. Other sediment samples were autoclaved and rinsed with lake water. Untreated surface sediment served as the control.

Sediment preparations were placed in a 50 × 15 × 10-cm plexiglass trough in sufficient quantity to cover the bottom to a depth of 2 cm. Each sediment type was adjacent to the other so as to form a continuous cover over the bottom. Lake water (1 liter) was slowly added from one end so that the sediment was undisturbed and 35 *Hyalella* were evenly distributed into water over the sediment. The trough was left for 24 hr in the dark before amphipods in each sediment type were enumerated.

To examine the importance of algae in determining the growth of *Hyalella*, *Chara* with attached epiphytes was collected from around springs in Marion Lake. This food source was rich in living microflora and assimilated with over 65% efficiency, in contrast to surface sediment, which *Hyalella* digested with 10–15% efficiency (Hargrave, 1970). Amphipods were collected, divided, and weighed immediately to give an initial estimate of the weight-frequency distribution and establish the reproducibility in subdividing the population. The remaining two groups of amphipods were placed in culture dishes with 200 ml of lake water. One group was offered *Chara* with epiphytes (10 g wet weight); the other was provided with samples of surface sediment (5–10 g wet weight). The cultures were held at 8 C on a 12-hr light–dark cycle and renewed every 4 days, when fresh *Chara* and sediment were added. After 30 days amphipods from both

culture dishes were removed, dried, and weighed. Growth rates were calculated by comparing amphipod mean dry weight at the beginning and end of the 30-day period.

RESULTS

DISTRIBUTION

The restricted depth distribution of *Hyalella* (Hamilton, MS, 1965; Mathias, MS, 1967) has been apparent during the present study (Fig. 1). The marked decrease in density below 2–3 m was found in all samples taken during 1968. There was also a corresponding decrease in amphipod body size (dry weight) with increasing depth of water over the sediment (Fig. 1).

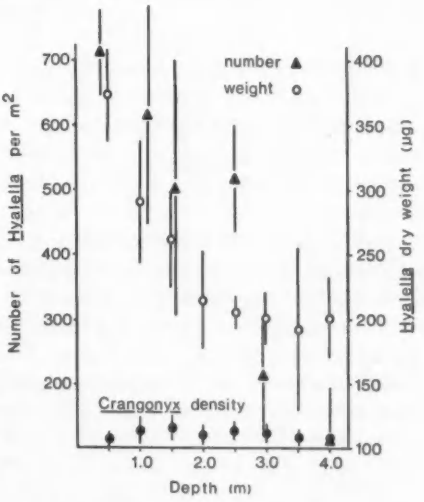


FIG. 1. Density and dry weight of adult *Hyalella azteca* at various depths in Marion Lake during May 1968. Density of *Crangonyx richmondensis occidentalis* also illustrated. All points are means of at least three determinations. Vertical lines represent standard errors of the mean.

The decrease in number and weight of *Hyalella* with increasing depth suggests a direct relation between amphipod dry weight and density. Intensive sampling at various depths during May 1968 showed such a relation to exist for densities up to 20 amphipods per Eckman sample; however, at higher densities dry weight and density appeared to be inversely related (Fig. 2). Examination of antennal segment number (21–25) and head length (0.55–0.66 mm), as described by Cooper (1965), showed that only adult *Hyalella* were present in the population. Thus, differences in mean body weight at various depths do not reflect a shift in age structure but indicate adults of a smaller size with increasing depth of water.

PHYSICAL FACTORS

The differences in size and density of *Hyalella* observed with depth in Marion Lake could be due to many factors. Physical conditions seem to be of little importance. *p*H is relatively constant and ranges from 6.6 to 6.9

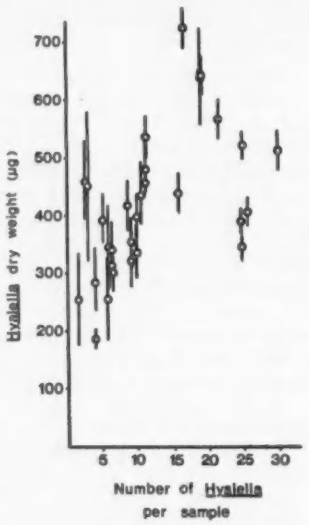


FIG. 2. Relationship between density and dry weight of adult *Hyalella azteca* in Marion Lake during May 1968. Points are individual samples, vertical lines indicate standard error of mean amphipod dry weight per sample.

in surface sediment from various depths; in 1968 the well-mixed water column supplied oxygen to all depths at near saturation levels, and temperature differences seldom exceeded 5 C (Hargrave, 1969).

The sediments in Marion Lake are a gyttja, which contain 35–45% organic matter (loss in weight after 1 hr at 600 C) and consist of aggregates of colloidal particles. There was a significant difference in total organic matter in surface sediment (top 1 cm) only in sediment covered with more than 4 m of water:

Depth (*m*)	0–1	1–2	2–3	3–4	4–5	5–6
Organic matter (%)	37.8	37.9	37.7	37.7	38.4	41.5
± SE	0.3	0.2	0.3	0.3	0.4	1.2

Both the color and texture of sediment varied with depth. Sediment collected in deep water contained fragments of leaves and bark and was dark brown in color and of fine silty consistency, whereas shallow-water sediment was a light brown color and much more clumped. The texture of shallow-water sediments was largely due to the distinct algal-mat layer and numerous chironomid tubes, which served to form a spongy semisolid mud–water interface. The algal-mat layer and network of chironomid tubes was much reduced in sediments below 3 m.

Numerous workers have stressed the importance of cover in influencing spatial differences in numbers of benthic organisms. In Marion Lake, *Hyalella* occurs largely on open sediment and does not show any contagious distribution associated with beds of *Potamogeton* or *Chara*. The density and mean dry weight of amphipods taken from these areas were not significantly different from samples taken on adjacent open sediment:

	Chara	*Potamogeton*	Sediment
Hyalella dry weight ($\mu g \pm SE$)	289±32	310±21	290±19
No./m²	310	345	335

The decrease in numbers and size of *Hyalella* with increasing depth of water over the sediment parallels the attenuation of light with increasing depth. Laboratory and field observations have shown that in Marion Lake an average of 80% of the amphipod population is within the sediment (D. Ware, personal communication) and this proportion is affected by light and temperature. Observations during the present study confirmed the tendency for amphipods to align themselves against a surface or under objects such as pieces of leaf.

PREDATION AND MIGRATION

If the factors of natality, mortality, and movement operate in a nonrandom way, changes in population size and structure may vary in different areas. In Marion Lake, trout (*Salmo gairdneri* Richardson), kokanee (*Oncorhynchus nerka* Walbaum), and salamanders (*Ambystoma gracile* Baird and *Taricha granulosa* Skilton) feed on *Hyalella* (Hamilton, MS, 1965). There is, however, at present no evidence to suggest that these predators are more numerous in deeper water. In addition, Mathias (MS, 1967) showed that instantaneous mortality rates of adult *Hyalella* in shallow and deep water were similar and egg and young mortality ranged from 70 to 80% in shallow and deep water, respectively. Thus, if this mortality is primarily due to predation, the effects appear to be similar at all depths.

Movement behavior of both species of amphipods in Marion Lake is currently being studied (A. Bryan, personal communication). Laboratory and field observations have suggested that amphipods move extensively over an area of a few meters distance, but there is little evidence of movement from shallow to deep water. Mathias (MS, 1967) suggested that a sudden increase and decrease in numbers of *Hyalella* in deepwater sediments during August 1966 was due to immigration from shallow water followed by emigration. These differences were not observed during the present study, but it is likely that the 3- to 4-week sample interval was too large to detect such shifts in population density, if they do occur.

Changes in spatial distribution of *Hyalella* might occur by means of swimming activity. *Hyalella* were never found in samples taken by towing a fine-mesh plankton net at various depths during the night and day on several occasions during 1967 and 1968. Also, zooplankton samples taken during 1967 by a high speed suction pump (McQueen, 1969) contained no *Hyalella*. If movement occurs on or close to sediment surfaces, however, these sampling methods would not be adequate.

FOOD SUPPLY

In Marion Lake, *Hyalella* is predominantly a deposit feeder, which digests algal and bacterial cells from ingested surface sediment material (Hargrave, 1970). *Chara* and epiphytes are also extensively browsed and dead leaf material

is ingested. *Hyalella* is also reported, however, to feed on freshly killed animal flesh of various types, attack wounded isopods, and eat its own dead (Embody, 1912). These observations could not be substantiated during the present study. Dead specimens of both *Crangonyx* and *Hyalella* were offered as the only source of food to freshly collected *Hyalella*. No evidence of ingestion or egestion was found after 3 weeks.

Possible correlation between *Hyalella* density and sediment organic material available for digestion was examined in two ways. During 1968, primary production by epibenthic algae on sediment at different depths in Marion Lake was measured (Hargrave, 1969) and compared with total numbers of *Hyalella*:

Depth (*m*)	0–1	1–2	2–3	3–4	4–5
Epibenthic primary production (*g C/m² per year*)	47.9	41.5	31.0	28.1	27.0
Total mean density of *Hyalella*/m² × 10^3	37.5	12.0	4.0	0.9	0.8

Amphipod density calculations are similar to those made by Hamilton (MS, 1965) and Mathias (MS, 1967). Both *Hyalella* numbers and epibenthic algal production decreased in a nonlinear manner with increasing depth of water over the sediment. The decline in amphipod numbers, however, was much greater than that observed in primary production. Measures of epibenthic primary production consider total algal productivity and do not indicate important species differences that occur at various depths. For example, in September 1968, diatoms were numerous on sediments covered with 1–2 m of water. Above and below this depth the standing crop of diatoms declined (G. Gruendling, personal communication). Similar differences have been found in spatial distribution of green and blue-green algae. In laboratory experiments (Hargrave, 1970), *Hyalella* digested different species of sediment microflora with different degrees of efficiency. Diatoms were digested readily (70%), but the blue-green alga *Anabaena* sp. was not (15%). These differences in assimilation indicate that not all epibenthic algal production is available to *Hyalella* and illustrate the difficulty of comparing amphipod biomass with total epibenthic algal production.

Sedimentary chlorophyll and ligninlike (acid-insoluble) material were measured in three replicate (2) sediment cores taken from depths at which amphipod samples were taken for comparison with amphipod distribution (Fig. 3). Although total organic matter in sediment above 4-m depth showed little variation with depth, both sedimentary chlorophyll and lignin content showed the largest gradients within the upper 2 cm. *Hyalella* showed a similar vertical distribution.

In both substrate-choice experiments, sediment to which diatoms were added contained the greatest percentage of *Hyalella* (Fig. 4). There was no significant difference between the numbers found in the sterilized sediments ($D_{max} = 24.5$; $P = 0.245$), as evaluated by a Kolmogorov-Smirov one sample test for goodness of fit (Siegel, 1956). Amphipods in diatom-enriched and natural

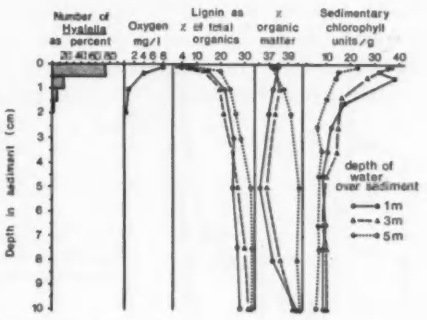


FIG. 3. Vertical distribution of *Hyalella azteca*, dissolved oxygen concentration, lignin, total organic matter, and sedimentary chlorophyll in sediment cores from various depths in Marion Lake. The average of duplicate samples taken during July 1968 is illustrated.

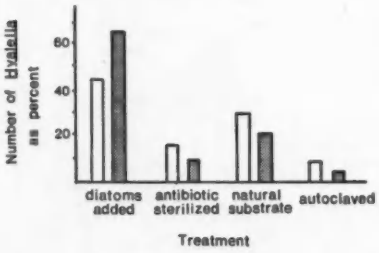


FIG. 4. Duplicate substrate-choice experiments to consider the distribution of *Hyalella azteca* after 24 hr in sediments treated in various ways to alter microflora content.

sediment also had darker gut contents than those in sterilized sediments, indicating reduced ingestion in the latter.

The antennae, which bear many small club-shaped appendages, appear to be important in *Hyalella*'s response to the sediment. The bristles on the antennal flagella have been shown to function as taste organs (Waterman, 1961) and this was apparent during feeding activity. The antennae continually move and touch the sediment when *Hyalella* is not feeding, but while it is browsing they are stationary on the sediment surface.

GROWTH RATE

During its 12- to 16-month life span, *Hyalella* grows from an egg (dry weight 14 μg) to a mature adult (dry weight 700–1000 μg) (Fig. 5). Differences in sizes of amphipods taken from different depths in Marion Lake were apparent throughout 1967 and 1968 (Fig. 5). Growth rates of young and adult *Hyalella*, as derived from changes in body dry weight during 1968, are presented in Fig. 6. Growth rates of juveniles are underestimated because of continued recruitment during the summer months. Egg production ceased during September, however, and growth rates thereafter are not affected by this error. Growth rates estimated from changes in biomass of the natural population will also be underestimated to the extent that larger amphipods are preferentially preyed upon (size-specific mortality). Mathias (MS, 1967) showed that instantaneous mortality rates of adult *Hyalella* in shallow and deep water are similar. Thus, if size-specific mortality occurs, as found by Cooper (1965) and D. Ware (personal communication), it appears to act equally at all depths

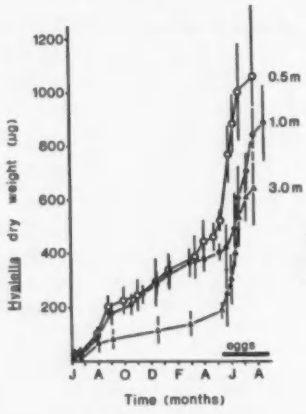
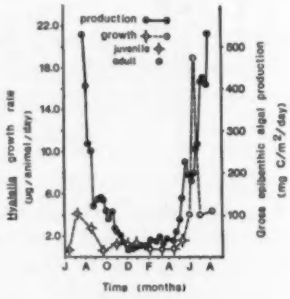


FIG. 5. Growth curves for *Hyalella azteca* at various depths in Marion Lake during 1968. Points are means of three determinations. Vertical lines indicate standard error of mean.

FIG. 6. Comparison of growth rate of *Hyalella azteca* and epibenthic primary production on sediment at 1.0-m depth in Marion Lake during 1968. Juvenile and adult growth rates shown separately.



in Marion Lake. Evidence presented above suggests that amphipods do not migrate between shallow and deep water.

Adult *Hyalella* showed maximum increments in biomass during June at all depths, but growth was greatest in shallow-water areas (Fig. 5, 6). Since temperature during this period was approximately 15 C at all depths and maximum summer temperatures (24–26 C) were not reached until late July (Hargrave, 1969), differences in growth rates observed during June must be due to other factors.

Changes in epibenthic primary production between April and June compare closely to increased *Hyalella* growth rate during this period (Fig. 6). During July, however, although primary production continued to increase, growth of adult amphipods decreased. This may be due to size-specific predation on larger amphipods and physiological senescence of old adult amphipods, which have lower feeding rates and appear generally less active than younger adults (B. T. Hargrave, unpublished data).

In laboratory experiments to examine the effect of diet on the growth of *Hyalella*, initial samples of the *Hyalella* population had mean dry weights ($\pm$SE) of 346 $\pm$ 25 μg and 355 $\pm$ 20 μg, which were not significantly different ($P < 0.05$) (Fig. 7A). After 30 days, amphipods held on *Chara* and epiphytes

weighed 554 ± 31 μg and those on sediment weighed 383 ± 27 μg (Fig. 7B, C). Thus, growth on *Chara* and epiphytes was 6.9 μg/animal per day, and on sediment it was 1.2 μg/animal per day. Similar experiments in June showed similar differences in growth — 8.8 μg/animal per day on *Chara* and epiphytes, 2.1 μg/animal per day on sediment.

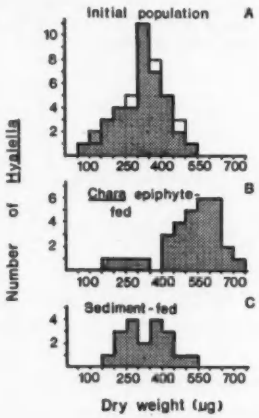


FIG. 7. The effect of diet on the growth of *Hyalella azteca*. Initial samples (A) taken from the amphipod population in Marion Lake on May 2, 1968. Replicate samples of amphipods fed *Chara* and epiphytes (B) and surface sediment (C) and weighed after 30 days. Temperature, 8 C.

Mortality rates of amphipods held under laboratory conditions were high (22% on *Chara* and 52% on sediment, Fig. 7). The reduced survival of *Hyalella* on sediment may have been affected by handling; these amphipods had to be transferred individually, whereas those clinging to *Chara* stalks were transferred by shaking the *Chara* into the culture. Although dead amphipods were not weighed, visual observation showed that all sizes of animals died. Thus, changes in mean body weight are not due to size-specific mortality.

SEASONAL ABUNDANCE

The density of *Hyalella* in Marion Lake changed markedly during 1968 (Fig. 8). Similar changes were noted by Hamilton (MS, 1965) and Mathias (MS, 1967). Mortality of subadult amphipods was high during the winter and spring, but the most abrupt change in abundance was during July and August. Peak periods of egg production occurred during late May and July, but maximum population size was not reached until mid-August.

The decrease in numbers and body size of *Hyalella* with increasing depth in Marion Lake is reflected in marked differences in egg production at various depths. Epibenthic algal production (Hargrave, 1969) shows a similar decrease with depth, although as mentioned above, the changes are not so marked:

Depth (*m*)	0–1	1–2	3–4
Eggs produced per m^2	7100	6450	350
Epibenthic primary production ($g\ C/m^2$) during period of egg production	34.0	30.0	20.8

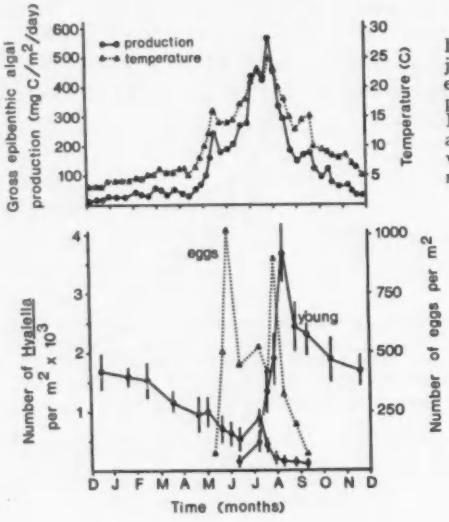


FIG. 8. Comparison of numbers of eggs, juveniles, and adult *Hyalella azteca* with epibenthic primary production and temperature on sediment at 1.0-m depth in Marion Lake during 1968. Numbers of amphipods are means of triplicate samples, vertical lines represent standard error of mean. Adults (open circles) occur from June until September.

Of the total eggs produced by the *Hyalella* population in shallow water, only 33% survived as juveniles after recruitment ceased in mid-September (Fig. 8). Similar mortality rates of eggs and juveniles were observed by Mathias (MS, 1967) who estimated that only 10% of the mortality occurred as eggs carried by females.

Increased epibenthic primary production during June in Marion Lake was paralleled by similar increases in *Hyalella*'s growth rate (Fig. 6). Since temperature was relatively constant (15 C, Fig. 8), changes in growth rate might be associated with increased epibenthic algal production. Brood size is also correlated with biomass, however (Fig. 9). A relation between body size and egg production has been observed previously (Wilder, 1940; Cooper, 1965), but the nonlinear nature of the relation has not been reported.

DISCUSSION

Hyalella azteca is widely distributed throughout lakes, streams, and ponds in North America in which the mean monthly temperature during the summer exceeds 10 C (Pennak, 1953; Bousfield, 1958). However, its local distribution may be restricted. *Hyalella* is generally found in shallow-water sediments, where it may be a dominant bottom organism, but it is seldom found below 2–3 m in depth (Jackson, 1912; Smith, 1952; Cooper, 1965). E. C. Bovee (personal communication) found *Hyalella* associated with the green alga *Cladophora fracta* only at a depth of 1 m. Within many shallow-water habitats its distribution shows an association with aquatic plants such as *Chara*, *Elodea*, and *Myriophyllum* (Cooper, 1965; Schneider, 1965).

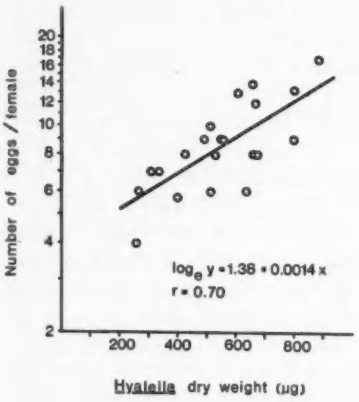


FIG. 9. Relationship between brood size and dry weight of *Hyalella azteca*.

The present study has shown that *Hyalella* has the highest density and largest body size in shallow-water sediments of Marion Lake. Density, growth, and egg production at various depths are correlated with measures of epibenthic algal abundance and production, which supports the view that sediment microflora is important in the nutrition of this deposit-feeding amphipod. In addition, substrate-choice experiments demonstrate that *Hyalella* is able to detect and ingest sediments containing diatoms.

The relationship between sediment microflora production and amphipod growth and distribution, however, is not clear-cut. In laboratory experiments, for example, epiphytes associated with *Chara* are a better source of food for growth than is sediment microflora. Yet, in Marion Lake, where *Chara* beds with abundant epiphytes occur around springs, *Hyalella* is neither more numerous nor larger in size than amphipods found on adjacent open sediment. Also, the mean dry weight of adults decreased at the highest densities observed in shallow-water sediments (Fig. 2). Since epibenthic algal production was maximal in these areas, amphipod size would be expected to be correspondingly high, if this food source is the only factor that determines amphipod body size.

The importance of cover in determining the distribution of benthic invertebrates has been considered by many workers (e.g., Andrews and Hasler, 1943; Eggleton, 1952; Rosine, 1955; Gerking, 1962; McLachlan, 1969). In general, areas with greater amounts of vegetation are found to contain higher numbers and biomass of many invertebrates, but this was not found with *Hyalella* in Marion Lake.

Cooper (1965) found that predation by perch and bluegills on *Hyalella* in Sugarloaf Lake was size-specific and only large amphipods were eaten. Work currently in progress at Marion Lake (D. Ware, personal communication) has also shown that rainbow trout ingest large *Hyalella*, but only if amphipods are visible on the surface of the sediment.

Seasonal changes in numbers of juvenile and adult amphipods found by Mathias (MS, 1967) and during this study show that predation is the most likely cause of observed mortality. Approximately 70% of *Hyalella*'s offspring are lost before recruitment ceases in September. This effect is of major importance in determining the population size during the subsequent year.

The movement of *Hyalella* will affect both its own production and the transfer of energy through predation. Previous studies have shown a marked negative phototactic response (Holmes, 1901; Phipps, 1915) and a high degree of thigmotaxis (Jackson, 1912). These responses probably determine *Hyalella*'s vertical distribution in the sediment. Some degree of isolation between shallow- and deep-water populations must occur to maintain the stable depth distribution of *Hyalella*, which in Marion Lake parallels light penetration. Light could be related to amphipod distribution indirectly through food supply. Although *Hyalella*'s food habits are described as omnivorous by Pennak (1953), algae and certain freshwater plants are extensively grazed (Embody, 1912; Jackson, 1912; Gaylor, 1922). *Hyalella*'s ability to digest sediment microflora has been demonstrated (Hargrave, 1970) and in this study growth rates of amphipods fed epiphytes were higher than those observed when sediment and microflora were consumed. Substrate-choice experiments demonstrate *Hyalella*'s ability to select sediments containing viable microflora, a characteristic of many benthic deposit-feeding invertebrates (Wilson, 1955; Meadows, 1964; Marzolf, 1965; Gray, 1967).

The vertical distribution of *Hyalella* in sediment in Marion Lake, although correlated with the maximum concentration of sedimentary chlorophyll at the sediment surface, is also related to the depth of oxygen penetration. In Marion Lake, *Hyalella* only occur in soft ooze sediments and are absent from patches of exposed gravel near inlet and outlet streams. Associations of *Hyalella* with soft mud bottoms and algal mats are also apparent from habitat descriptions given by Jackson (1912), Cooper (1965), and Bovee (1949, and personal communication). Thus *Hyalella*'s vertical position in the sediment may be determined by the combined effects of its negative phototaxis, thigmotaxis, and the depth of oxygen penetration.

Laboratory experiments have shown that *Hyalella*'s growth is affected by the amount of microflora in the diet. Thus, the reduced size of amphipods in deepwater sediments in Marion Lake might be related to lower rates of epibenthic algal production. Many factors can affect the growth rate of an individual in a population. Temperature (Bovee, 1949; Geisler, 1944), the type and quantity of food, CO_2 and O_2 concentration, and population density (Wilder, 1940) have all been demonstrated to influence growth rate in *Hyalella* under laboratory conditions. These laboratory-determined growth rates, however, may not be realistic measures of growth under natural conditions. Comparative growth rates may be determined by observing increments in biomass in a natural population.

The maximum growth rate of amphipods held in the laboratory was 8.8 μg/amphipod per day, less than half the maximum growth rate observed in

amphipods taken from the field (Fig. 6); although laboratory experiments were conducted at 8 C, and temperatures in the field were 15 C, these differences are probably not entirely due to temperature. Even though food was renewed every 4 days, *Hyalella* in the culture had consumed most of the *Chara* epiphytes and sediment particles had been converted to faeces. *Hyalella*'s growth rate may vary over a 20-fold range under natural conditions (Fig. 6) and food supply appears to have a marked effect.

Hyalella's egg production and juvenile recruitment were delayed 1 or 2 weeks in deepwater sediments in Marion Lake (Mathias, MS, 1967). This may be due to temperature (Cooper, 1965; Mathias, MS, 1967). In Marion Lake, young recruited during the summer do not mature until the next year (Fig. 8). This is in contrast to Cooper's (1965) study where *Hyalella* juveniles matured and reproduced during their first summer. The difference is probably also due to temperature effects on maturation time. This was calculated as 98 days at 15 C and 36 days at 20 C by Cooper (1965). Mean summer temperature in Marion Lake during 1968 was 17 C; Cooper reported a mean temperature of 21.5 C for June, July, and August in his study area.

The combined effects of temperature and food supply on developmental and maturation time might account, in part, for the smaller sizes of *Hyalella* in deepwater sediments in Marion Lake (Fig. 1). This suggestion is supported by the close correlation between the onset of egg production in May and increased temperature and epibenthic algal production (Fig. 8). As expected, both temperature and epibenthic primary production are inversely related to depth of water over the sediment (Hargrave, 1969). In addition, the relation between body size and egg production indicates that eggs are produced at a rate dependent upon the growth and mortality rates of adult amphipods. Thus, although temperature influences the rate of egg development, increased epibenthic algal production during April, May, and June may supply the energy necessary for growth and subsequent egg production. This would also account for higher numbers of amphipods in shallow-water sediments of Marion Lake. If size-specific mortality occurs, large adult amphipods are removed from the population. This would result in a population of adults with smaller body size and fewer eggs per female.

The above discussion on the size-frequency distribution of *Hyalella* with depth is made in the absence of adequate data on movement and the effects of predation on *Hyalella* at various depths in Marion Lake. Such information will contribute a great deal to an understanding of *Hyalella*'s role in the lake ecosystem. At present, however, predation seems the most likely cause for the rapid decline in numbers of juveniles during the summer, since epibenthic algal production is maximal at this time.

ACKNOWLEDGMENTS

This work was supported by the National Research Council of Canada, for which I am grateful. Dr I. E. Efford made many useful suggestions and provided both valuable discussion and enthusiasm throughout the research. Dr E. C. Bovee, The University of Kansas, Lawrence,

Kansas, kindly provided an unpublished bibliography of papers concerned with the natural history and ecology of *Hyalella azteca*. Mr D. Ware read the manuscript and offered useful criticism.

REFERENCES

ANDERSON, R. O. 1959. A modified flotation technique for sorting bottom fauna. Limnol. Oceanogr. 4: 223–225.

ANDREWS, J. D., AND A. D. HASLER. 1943. Fluctuations in the animal populations of the littoral zone of Lake Mendota. Trans. Wis. Acad. Sci. Arts Lett. 35: 175–186.

BOUSFIELD, E. L. 1958. Fresh-water amphipod crustaceans of glaciated North America. Can. Field Natur. 72: 55–113.

BOVEE, E. C. 1949. Studies on the thermal death of *Hyalella azteca* (Saussure). Biol. Bull. (Woods Hole) 96: 123–128.

COOPER, W. E. 1965. Dynamics and productivity of a natural population of a fresh-water amphipod, *Hyalella azteca*. Ecol. Monogr. 35: 377–394.

EFFORD, I. E. 1967. Temporal and spatial differences in phytoplankton productivity in Marion Lake, British Columbia. J. Fish. Res. Bd. Canada 24: 2283–2307.

EGGLETON, F. E. 1952. Dynamics of interdepression benthic communities. Trans. Amer. Microsc. Soc. 71: 189–228.

EMBODY, G. C. 1912. A preliminary study of the distribution, food and reproductive capacity of some freshwater amphipods. Int. Rev. Ges. Hydrobiol. (Suppl.) 4: 1–33.

GAYLOR, D. 1922. A study of the life history of *Hyalella knickerbockeri* Bate. Proc. Indiana Acad. Sci. 1922: 239–250.

GEISLER, F. S., SR. 1944. Studies on the postembryonic development of *Hyalella azteca* (Saussure). Biol. Bull. (Woods Hole) 86: 6–22.

GERKING, S. D. 1962. Production and food utilization in a population of bluegill sunfish. Ecol. Monogr. 32: 31–78.

GRAY, J. S. 1967. Substrate selection by the archiannelid *Protodrilus hypolecus* Armenante. J. Exp. Mar. Biol. Ecol. 1: 47–54.

HAMILTON, A. L. MS, 1965. An analysis of a freshwater benthic community with special reference to the chironomidae. Ph.D. Thesis. Univ. British Columbia, Vancouver, B.C. 216 p.

HARGRAVE, B. T. 1969. Epibenthic algal production and community respiration in the sediments of Marion Lake. J. Fish. Res. Bd. Canada 26: 2003–2026.

1970. The utilization of benthic microflora by *Hyalella azteca* (Amphipoda). J. Anim. Ecol. 39: 427–437.

HOLMES, S. H. 1901. Phototaxis in the amphipoda. Amer. J. Physiol. 5: 211–234.

JACKSON, H. H. T. 1912. A contribution to the natural history of *Hyalella knickerbockeri* Bate. Bull. Wis. Natur. Hist. Soc. 10: 49–60.

LOVEGROVE, T. 1966. The determination of the dry weight of plankton and the effect of various factors on the values obtained, p. 429–467. *In* H. Barnes [ed.] Some contemporary studies in marine science. George Allen and Unwin Ltd., London, England.

MARZOLF, G. R. 1965. Substrate relations of the burrowing amphipod *Pontoporeia affinis* in Lake Michigan. Ecology 46: 579–592.

MATHIAS, J. A. MS, 1967. Population energetics of two amphipod species in Marion Lake. M.Sc. Thesis. Univ. British Columbia, Vancouver, B.C. 74 p.

MCLACHLAN, A. J. 1969. The effect of aquatic macrophytes on the variety and abundance of benthic fauna in a newly created lake in the tropics (Lake Kariba). Arch. Hydrobiol. 46: 212–231.

MCQUEEN, D. J. 1969. Reduction of zooplankton standing stocks by predaceous *Cyclops bicuspidatus* Thomasi in Marion Lake, British Columbia. J. Fish. Res. Bd. Canada 26: 1605–1618.

MEADOWS, P. S. 1964. Experiments on substrate selection by *Corophium* species: films and bacteria on sand particles. J. Exp. Biol. 41: 499–512.

PENNAK, R. W. 1953. Fresh-water invertebrates of the United States. Ronald Press, New York, N.Y. p. 435–446.

PHIPPS, C. F. 1915. An experimental study of the behavior of amphipods with respect to light. Biol. Bull. (Woods Hole) 28: 120–223.

ROSINE, W. N. 1955. The distribution of invertebrates on submerged aquatic plant surfaces in Muskee Lake, Colorado. Ecology 36: 308–314.

SCHNEIDER, J. C. 1965. Further studies on the benthic ecology of Sugarloaf Lake, Washtenaw County, Michigan. Pap. Mich. Acad. Sci. Arts Lett. 50: 11–29.

SIEGEL, S. 1956. Nonparametric statistics for the behavioral sciences. McGraw-Hill, New York, N.Y.

SMITH, M. W. 1952. Limnology and trout angling in Charlotte County lakes, New Brunswick. J. Fish. Res. Bd. Canada 8: 383–452.

WATERMAN, T. H. 1961. The physiology of Crustacea. Vol. II. Sense organs, integration and behavior. Academic Press, New York, N.Y.

WIESER, W. 1956. Factors influencing the choice of substratum in *Cumella vulgaris* Hart (Crustacea, Cumacea). Limnol. Oceanogr. 1: 275–285.

WILDER, J. 1940. The effects of population density upon growth, reproduction and survival of *Hyalella azteca*. Physiol. Zool. 13: 239–461.

WILSON, D. P. 1955. The role of micro-organisms in the settlement of *Ophelia bicornis* Savigny. J. Mar. Biol. Ass. U.K. 34: 531–543.

Effects of Transition Metal Ions on the Extractable Protein of Fish Muscles

C. H. CASTELL, BARBARA SMITH, AND WANDA NEAL

Fisheries Research Board of Canada
Halifax Laboratory, Halifax, N.S.

CASTELL, C. H., B. SMITH, AND W. NEAL. 1970. Effects of transition metal ions on the extractable protein of fish muscles. J. Fish. Res. Bd. Canada 27: 701–714.

The addition of 10–50 ppm of several transition metal ions rapidly decreased the extractable protein content of blended fish muscle. The most active metals were Cu^{++} and Cr^{++} followed by Sn^{++} and Cd^{++}. Other ions tested, Ce^{++}, Zn^{++}, Ni^{++}, Co^{++}, Fe^{++}, Fe^{+++}, V^{++}, and Mn^{++}, brought about relatively little or no change in extractable protein nitrogen (EPN) values. Effects of the metals differed with muscle from different species. Lean fish were more susceptible than either fatty fish or shellfish. Of all the species tested the metals had the least effect on scallops. A limited number of tests indicated that fish that were badly emaciated through starvation were more susceptible to the metal-induced change than normal individuals of the species.

Addition of certain amino acids, notably cysteine, histidine, glutamic acid, and tryptophane, protected the muscle against metal-induced reduction of EPN. With lean fish, the amino acids that protected the muscle against metal-induced change in the protein also protected it against metal-induced oxidation of the lipids; with fatty fish, protection against rancidity was somewhat less; the invertebrates seemed to be normally protected against both without the addition of amino acids. This natural immunity of marine invertebrate muscle to changes induced by the addition of heavy metal ions may in part be attributed to their normally high content of free amino acids.

Received October 30, 1969

INTRODUCTION

EFFECTS OF transition metal ions on the deterioration of stored fish and meat is usually attributed to their ability to catalize oxidation of unsaturated lipids with the development of rancidity. However, in addition to their catalytic effect on the lipids the metal ions also react with other components of these complex foods. In terms of deterioration of the food product this can have two effects: their reactions with nonfatty components may bring about other types of deleterious change; or, the metals may be bound up in such a manner that they can no longer act as lipid oxidation catalysts, in which case the development of rancidity will be inhibited. This is one reason why the capacity of metal ions to produce rancidity in an extracted oil may be different from their ability to oxidize the same oil in the flesh before it has been extracted (Lea, 1952). It is also a reason for the difference in the ability of metal ions to produce rancidity when they have been added to the flesh of different species of fish and other marine animals (Castell and Spears, 1968). Even if one's interest is wholly confined to rancidity, the picture can never be complete until consideration is given to the changes that are simultaneously taking place in the nonlipid components of the food.

Printed in Canada (J1636)

It has been known for some time that proteins, peptides, amino acids, porphyrins, and many other cell constituents form stable complexes with ions of transition metals (Albert, 1960). It is also known that some of these metal ions will bring about the formation of lipid–protein complexes when they react with phospholipids and soluble proteins (Fullington, 1967; Fullington and Hendrickson, 1966). There is reason to believe, therefore, that at least some of these metal ions may bring about changes in the solubility of fish muscle protein; and under certain conditions they may also be active in the formation of lipid–protein complexes that can significantly alter the texture of the muscle.

This paper deals with the ability of trace amounts (1–50 ppm) of 12 transition metal ions to bring about changes in fish muscle proteins as indicated by their decreased extractability in salt solution. It further tries to determine whether this reaction with the protein affects the capacity of these metal ions to catalyze oxidation of the lipids in the muscle, and whether the metal-induced changes in the protein show any considerable difference when the muscle is taken from different species of fish.

MATERIALS AND METHODS

Muscle from the following species was used: Atlantic cod (*Gadus morhua*), Atlantic haddock (*Melanogrammus aeglefinus*), American plaice or flounder (*Hippoglossoides platessoides*), Atlantic halibut (*Hippoglossus hippoglossus*), Atlantic wolffish or sea catfish (*Anarhichas lupus*), thorny skate (*Raja radiata*), Atlantic herring (*Clupea harengus harengus*), Atlantic mackerel (*Scomber scombrus*), Atlantic salmon (*Salmo salar*), lumpfish (*Cyclopterus lumpus*), lobster (*Homarus americanus*), queen crab (*Chionoecetes opilio*), and giant scallop (*Placopecten magellanicus*).

Most of the fish and other marine animals used in these experiments were obtained live from the station's aquaria. The muscles from these animals were in a prerigor condition when the experiments were carried out. Some tests were also made on fillets cut from iced fish that had passed through rigor, but before any perceptible bacterial deterioration had taken place. Muscle was prepared by blending whole fillets (in the case of fish) with twice their weight of water. Immediately after blending (always within 2 min), a metal salt solution was added to the blend and thoroughly mixed, giving a final concentration of 1 part of muscle to 3 parts of water. Metals were added as chlorides or sulphates, and recorded concentrations are in parts per million (ppm) of the metal component of the salt in the blend.

Soluble protein determinations were made by using the salt extraction method of Dyer et al. (1950, 1964), and colorimetric readings with the addition of biuret solution, following the procedure of Snow (1950). The results are expressed as percent extractable protein nitrogen (EPN) in the muscle. Special care was taken to prevent foaming during blending of the extracted protein solution. The muscle from some species produced a cloudy extract that interfered with the colorimetric reading. This was overcome by running one test with the normal biuret reagent and a second with the reagent minus copper sulphate, and then subtracting the values of the second test from the results of the first. This was used mainly with muscle from some fatty fish, but it was always employed where cloudy extracts were encountered.

The extent of lipid oxidation was measured by the thiobarbituric acid (TBA) reaction using the procedure of Tarladgis et al. (1960), and the results are expressed as milligrams of malonaldehyde per 1000 g of fish muscle. Where V^{++}, Fe^{++}, or Fe^{+++} had been added to the blend, and also with all tests made with dark muscled fish, such as mackerel or herring, the antioxidants EDTA and propyl gallate were added to prevent metal-induced oxidation of the lipids during distillation (Castell et al., 1966; Castell and Boyce, 1966). The fat content of the fish muscle was determined by the methanol–chloroform extraction method of Bligh and Dyer (1959).

EXPERIMENTAL RESULTS

Effects of Metal Ions on EPN of Cod and Scallop Muscles

The first tests were purposely carried out with relatively high concentrations of the metals so as to obtain a general picture of their relative activity. Fifty ppm of 12 metal ions were added to blends prepared from postrigor cod muscle and from scallop muscle that had been iced for approximately 24 hr after shucking. The tests were made after the blends, to which the metals had been added, had stood for 24 hr at 0 C. The results (Table 1) are similar

Table 1. Extractable protein values (as % EPN) of blended cod and scallop muscles after the addition of 50 ppm of 12 different heavy metal ions.

Added metal ion	Cod muscle	Scallop muscle
None	2.09	1.84
Mn^{++}	2.16	2.13
Fe^{+++}	2.07	1.84
Fe^{++}	2.12	1.84
V^{++}	2.06	1.95
Co^{++}	2.03	1.87
Ni^{++}	1.95	1.87
Zn^{++}	1.95	1.84
Ce^{++}	1.92	1.87
Cd^{++}	1.47	1.86
Sn^{++}	1.42	1.87
Cr^{++}	0.63	0.75
Cu^{++}	0.54	1.80

to those of other tests using the same concentration of these metals with other samples of cod and scallop muscle. With cod muscle Cu^{++} consistently brought about the greatest reduction in EPN ranging from 60 to 75%; Cr^{++} was next with reductions of 50–70%; Sn^{++} and Cd^{++} gave reductions of 25–35%; the remaining metals Ce^{++}, Zn^{++}, Ni^{++}, Co^{++}, Fe^{++}, Fe^{+++}, V^{++}, and Mn^{++}, brought little or no significant reductions. The addition of Mn^{++} always resulted in EPN values that were higher than those for the control samples.

The results with scallop muscle were not the same as those for cod. With the exception of Cr^{++} (about 60% decrease in EPN) none of the metals had much effect. Even Cu^{++} had little or no effect on the scallop muscle although it consistently brought about a large reduction in EPN for cod.

Effects of Ion Concentration on Cu^{++}-Induced Changes in EPN

Increasing concentrations from 1 to approximately 30 ppm brought about a steep decline in the curve showing the relationship in cod muscle

between EPN and the concentration of added Cu++ (Fig. 1); above 40 ppm there is another straight line relation, but this section of the curve is much less steep. This figure also shows the relative ineffectiveness of the same range of Cu++ concentrations for reducing the EPN of scallop muscle.

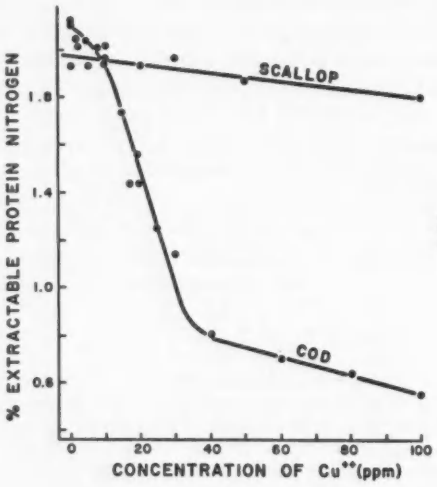


FIG. 1. Reductions in extractable protein in cod and scallop muscles with increasing concentrations of Cu++.

EFFECTS OF HOLDING TIME

Reaction between the added Cu++ and the proteins in the muscle was rapid. Several series of tests were made in which different concentrations of Cu++, between 15 and 50 ppm, were added to cod blend and then tested periodically for holding periods from 1 min up to 48 hr at 0 C. In every instance, minimum EPN values had been reached within 1 min after the Cu++ solution had been added to the blend, and the reaction may have almost instantly gone to completion. For convenience in arranging the experiments, 2 hr was used as a standard holding time for all subsequent tests dealing with the effect of Cu++ on EPN values. Also, in determining the effect of any reagents on the Cu++-induced changes in the protein, they were added to the blend before the metal salt solution.

EFFECTS OF AMINO ACIDS ON Cu++-INDUCED CHANGES IN EPN

The addition of some free amino acids protected blended cod muscle against Cu++-induced changes in EPN (Table 2). These amino acids also had an antioxidant effect when added to the blend, protecting it against the development of Cu++-induced rancidity. Inhibition by the sulphur-containing amino acids decreased with the degree of oxidation of the sulphur in these compounds (Fig. 2).

TABLE 2. Inhibition (%) of Cu^{++} (30 ppm)-induced changes in EPN and rancidity in blended cod muscle by 0.01 and 0.1 M concentrations of 17 amino acids.

	On decrease in EPN		On increase in rancidity	
Amino acid	0.01 M	0.1 M	0.01 M	0.1 M
Cysteine (free base)	73	100	85	100
Histidine	57	100	68	92
Homocysteine	37	65	70	100
Tryptophane	53	69	49	66
Threonine	–	58	–	34
Aspartic acid	24	31	75	98
Asparagine	None	39	52	67
Cystine	5	8	2	21
Proline	2	6	15	42
Phenylalanine	None	2	13	23
Valine	None	3	6	8
Tyrosine	None	None	None	3
Alanine	None	None	None	None
Leucine	None	None	3	37
Methionine	None	2	20	39
Glutamine	None	None	[a]	[a]
Taurine	None	None	None	None

[a]Rancidity increased instead of being inhibited when glutamine was added.

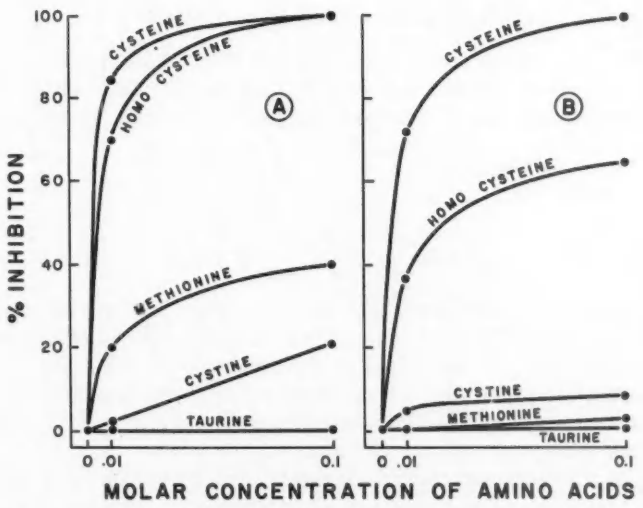


FIG. 2. Inhibition of Cu^{++}-induced rancidity (A) and Cu^{++}-induced decreases in extractable protein (B) in cod muscle by the addition of 0.01 and 0.1 M concentrations of sulphur-containing amino acids. The concentration of Cu^{++} added to the blend was 30 ppm.

These observations suggest that the amino acids complexed with the metal in a way that interfered with its catalytic activity. The chelating agents, EDTA and propyl gallate, also interfered with changes in EPN and the development of rancidity resulting from the addition of Cu^{++}:

	Fish alone	Fish + Cu^{++}	Fish + Cu^{++} + EDTA	Fish + Cu^{++} + propyl gallate
EPN (%)	2.17	0.81	2.06	1.64
Malonaldehyde value (*mg/kg*)	0.30	13.90	0.25	0.38

However, to be effective the EDTA must come in contact with the Cu^{++} before the metal ions have had an opportunity to react with the protein. This was shown by an experiment in which Cu^{++}, EDTA, and fish muscle were combined in different orders:

Mixed together	Cu^{++} + EDTA	EDTA + fish	Cu^{++} + fish	Fish alone	Fish + Cu^{++}	Fish + EDTA
Added 1 min later	Fish	Cu^{++}	EDTA	–	–	–
EPN (%)	1.87	1.77	1.16	1.94	0.90	1.90

One minute contact between the Cu^{++} and the muscle, before adding the EDTA, was sufficient to bring about a marked reduction in extractable protein, and this was not reversed by the chelating agent.

Effects of *p*H and Addition of Free Fatty Acids

Within the range usually encountered in fishery products (6.0–7.5) differences in *p*H of blended cod muscle had relatively little effect. Both with and without added Cu^{++}, increasing alkalinity brought about a slight decrease in EPN:

*p*H	6.0	6.5	6.8	7.0	7.2	7.5
No added Cu^{++}	1.94	1.97	1.82	1.89	1.79	1.61
With 20 ppm Cu^{++}	1.29	1.52	1.23	1.43	1.35	1.11

A wider range of *p*H values would probably show far greater changes.

Oleic and linoleic acids were added to blended cod muscle so as to give concentrations of 0.5% (in terms of the muscle or 0.125% in terms of the blend) followed by the addition of 30 ppm Cu^{++}. After a holding period of 2 hr the decreases in EPN values resulting from the Cu^{++} were the same for the samples with and without the added fatty acids.

It was also observed that for longer holding periods and especially if the blends were frozen and stored at −5 or −10 C, the addition of linoleic acid by itself brought about considerable reduction in EPN values. This was accompanied by pronounced changes in the physical condition of the blend especially in areas adjacent to the surface. These texture changes were not ob-

served where rapid reduction of the EPN was brought about by the addition of the heavy metal ions.

Effects of Cu^{++} on EPN of Muscle from Different Species

It has already been shown that increasing concentrations of Cu^{++} had a decidedly different effect on the EPN values of blends prepared from cod and scallop muscle (Fig. 1). This type of experiment was repeated using muscle from 11 species (Fig. 3). The difference in the initial EPN content of the

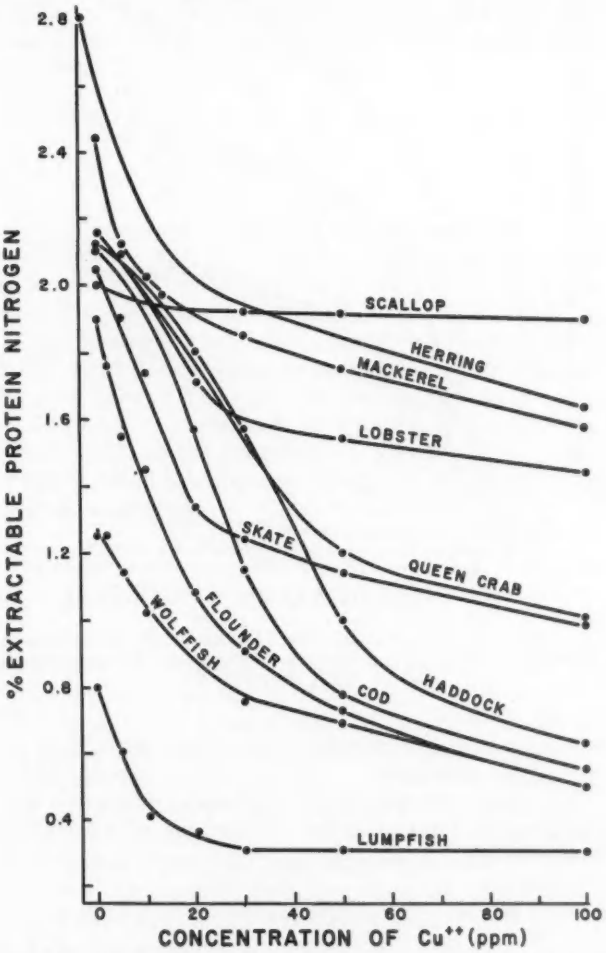


Fig. 3. Effects of adding increasing concentrations of Cu^{++} on the extractable protein of muscle from various species of marine fishes and shellfishes.

different species makes it somewhat difficult to compare the effect of the added Cu^{++} in this figure. To overcome this, the data were recalculated to show the percent decrease in EPN caused by the Cu^{++} for each species (Fig. 4).

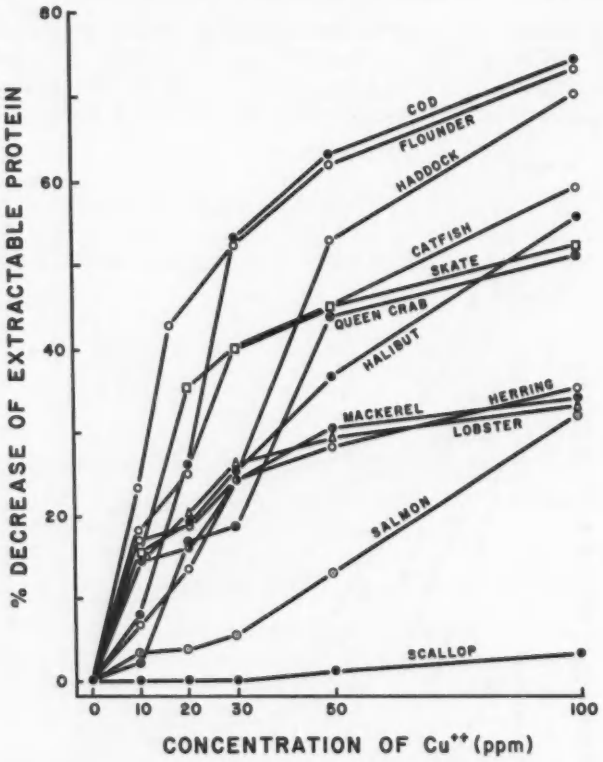


FIG. 4. Effects of increasing concentrations of Cu^{++} on the percent decrease in extractable protein in muscle from various species of marine fishes and shellfishes.

The question arises as to whether these curves are typical of the species they represent. The experiments were repeated with cod, haddock, herring, and scallops and the results were similar to those shown in the figure for these species, except for a few instances where muscle was taken from physiologically abnormal fish.

EFFECTS OF THE PHYSIOLOGICAL CONDITION OF THE FISH

There were exceptions to the statement that EPN – Cu^{++} concentration curves were typical for each species. When the fish were in an abnormal physiological condition the curves were quite different from those of the normal

fish. For example, tests were run on muscle from two Atlantic salmon. The first was a normal, healthy salmon and the second was a slinky, starved, spent female that had been in captivity for many months before it was killed. The lipid content of the fillet from the healthy fish was approximately 6% whereas that of the spent fish was 0.70%, indicating that the depot fat had been used up and that only the residual phospholipids remained. Although both were of the same species one was a fatty fish and the other had a fat content typical of the lean fish. The fillets cut from the spent fish were also much softer and more emaciated than those of normal fish. The effects of Cu^{++} on their EPN values was different. The normal salmon had high values, somewhat similar to that of the mackerel or herring; the values for the badly depleted salmon were below those of cod, haddock, or flounder:

Added Cu^{++} (*ppm*)	0	5	10	20	50	100	200
Normal salmon	2.62	2.49	2.53	2.57	2.27	1.76	0.98
Depleted salmon	1.21	1.15	1.05	0.90	0.69	0.62	0.40

In a few instances, where only one fish was available for testing, there is some doubt as to whether the curves are typical for the species. This particularly applies to the lumpfish (Fig. 4). At the time of slaughter the notes regarding this fish included the following: "Fish taken live from the aquarium; female with eggs almost mature; fillets jelly-like in appearance; fat content of muscle 1.3%; *p*H of blended muscle 7.5, which seems to be high for muscle immediately after death." The curve, therefore, may be lower than that of normal lumpfish taken at a period when they are not spawning.

These results indicate that normal spawning and feeding cycles may change not only the EPN content of the muscle but also the EPN – Cu^{++} concentration curve for a given species.

Relation between Rancidity Induced by Metal Ions and Induced Changes in EPN Values

Because two reactions occur at the same time it does not necessarily mean a causal relation between them. However, when the same catalyst is involved in both reactions such a relation becomes more probable. This is the case with metal ion-induced changes in the proteins and lipids of fish muscle. Two of the very active lipid oxidation catalysts, vanadium and iron, apparently have no effect on the extractable protein; copper, on the other hand, is involved in both reactions. The problem is more complicated because of the difference in the activity of specific metals in catalyzing rancidity and changing EPN values in muscle from different species of fish.

Where there was no reaction with the protein, as with Fe^{++}, the development of rancidity was very rapid (5 or 10 min); the Cu^{++}, on the other hand, had a long induction period of approximately 10–12 hr (Fig. 5). The curves for V^{++} were similar to those for Fe^{++}. Somewhat similar relations were shown to exist when EPN values and rancidity were plotted against the con-

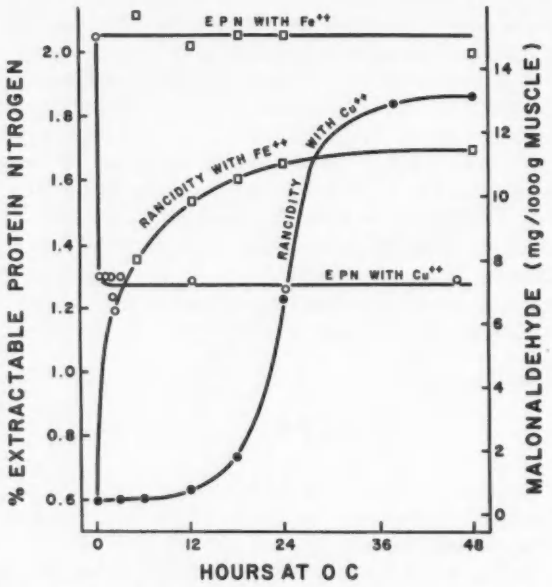


FIG. 5. Rates of development of rancidity and of decrease in extractable protein in cod muscle after the addition of 20 ppm Cu^{++} and of Fe^{++}.

centrations of added Fe^{++}, V^{++}, and Cu^{++} (Fig. 6). It requires much less Fe^{++} or V^{++} than Cu^{++} to produce rancidity. These results indicate that some component of the cod muscle has an inhibitory effect on the Cu^{++}-induced rancidity, but does not affect the Fe^{++} and V^{++}-induced rancidity; they further suggest that the reaction of the Cu^{++} with the proteins, resulting in decreased EPN, may be linked with this inhibition.

Similar experiments were carried out to determine the effect of Cu^{++} on the development of rancidity and changes in EPN for a number of species, including lean fish, fatty fish, Crustacea, and shellfish. For the other lean fish (haddock, pollock, flounder, and wolffish) the results were similar to those already given for cod. In each instance Cu^{++} brought about a considerable decrease in EPN and the induction period for the development of rancidity was relatively long. With these same lean fish Fe^{++} and V^{++} produced rancidity more rapidly and from lower concentrations than they did with Cu^{++}.

The rest of the species tested (Fig. 7) fell into two groups: the fatty fish in one, the lobster, queen crab, and scallops in the other.

Although Cu^{++} did reduce the EPN values of the fatty fish, for similar concentrations, they remained much higher than those of the lean fish. With the fatty fish, the long induction period with Cu^{++} was eliminated and rancidity developed at much lower Cu^{++} concentrations. These results indicate that, for fish with a high content of depot fat in the muscle, the extractable

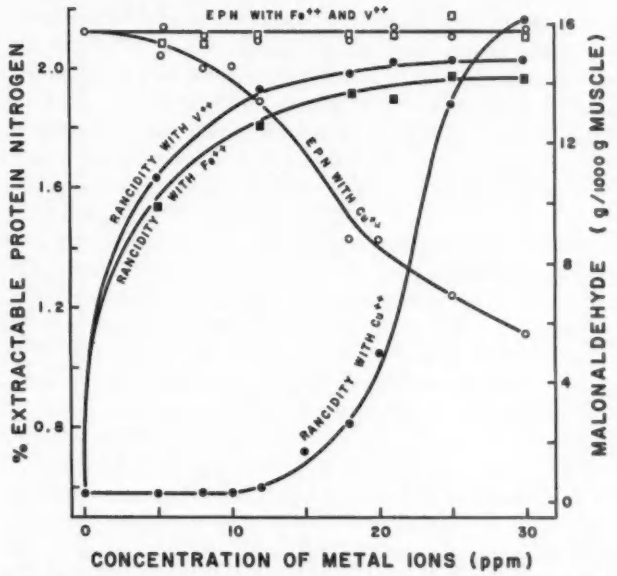


FIG. 6. Effects of increasing concentrations of Cu^{++}, Fe^{++}, and V^{++} on the development of rancidity and in the reduction in the amount of extractable protein in cod muscle.

protein exerts much less effect on the development of copper-induced rancidity than in the case of fish with a low lipid content.

The muscle of Crustacea has been shown previously to be very resistant to rancidity induced by metal ions, especially Cu^{++} (Castell and Spears, 1968). This has been confirmed by the present study and it has also been shown that lobster muscle is relatively resistant to Cu^{++}-induced changes in the EPN. The scallop muscle was similar to the lobster, except that it was slightly more resistant to changes in EPN and slightly less resistant to rancidity caused by the addition of Cu^{++}.

DISCUSSION

Perhaps the most important result from this study has been to reemphasize the interrelation between changes taking place in the lipid and in the protein compounds of the muscle. This is illustrated by comparing the effects on the muscle of adding trace amounts of Cu^{++} and Fe^{++}. Both are very active lipid oxidation catalysts. When added to extracted unsaturated lipids both rapidly accelerate the development of rancidity (Lea, 1938); when added to lean fish muscle, Fe^{++} rapidly produces rancidity, but with Cu^{++} there is quite a long induction period. Also, when added to muscle, it requires much larger concentrations of Cu^{++} than Fe^{++} to produce randicity.

These reactions become understandable when it is known that Cu^{++}, but not Fe^{++}, reacts with the protein in the muscle to form stable complexes;

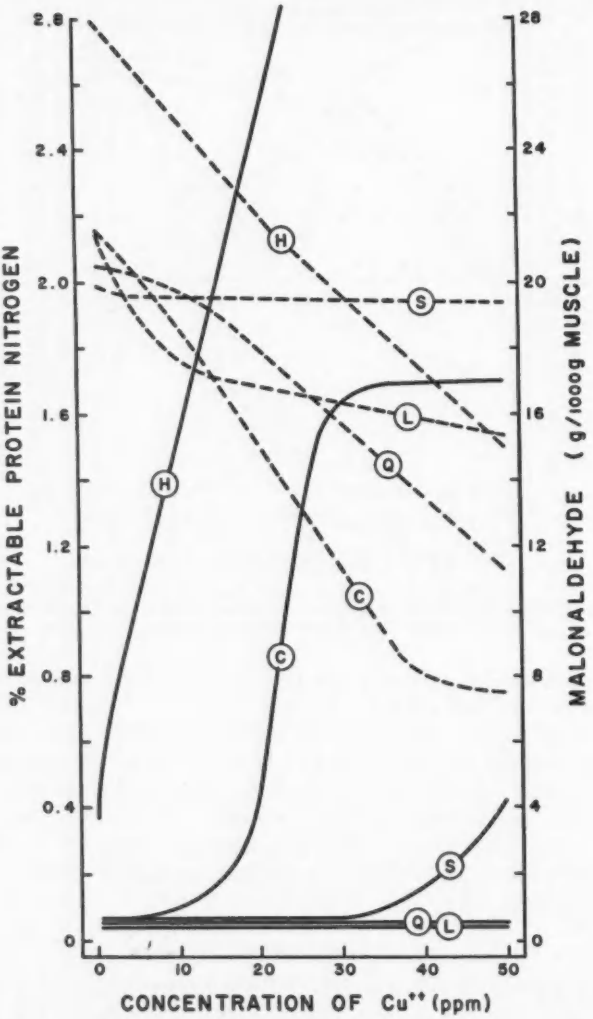


FIG. 7. Effects of Cu^{++} concentration on the development of rancidity (solid lines) and the reduction in the amount of extractable protein (broken lines) in muscle from cod (C), herring (H), scallops (S), queen crab (Q), and lobster (L).

and this interferes with its catalytic activity on the fat. Österberg (1967) studied metal ion complexes with peptides, and found that Fe^{++} belongs to that group of metals that bind to the anionic groups, forming relatively weak complexes; Cu^{++} belongs to another group that binds to sulphur, nitrogen, and oxygen, forming stable complexes. Cu^{++} itself is the most active of the

second group of metal ions, binding to sulphur in preference to nitrogen and oxygen. Frieden (1968) in discussing the biochemistry of copper, points out that it possesses, to a greater degree than any other metal, the property of reacting with amino acids and proteins to form very stable chelates. Studies made with amino acids, as well as a number of other complexing agents, have shown that the order of affinity of the metal ions to form complexes is similar for several different complexing agents, namely $Cu^{++} > Ni^{++} > Zn^{++} > Cd^{++} > Fe^{++} > Mn^{++}$ (Albert, 1950).

The inhibiting effect of certain amino acids on lipid oxidation has been known for many years (Lea, 1936, 1938). In a recent report dealing with the combined effect of amino acids and metal salts on lipid oxidation, Marcuse (1967) states that histidine and tryptophane are two of the most effective amino acids for inhibiting metal-catalyzed rancidity and he concludes that the mechanism of the inhibition is the formation of metal ion-amino acid chelates. In a previous paper in this series (Castell and Bishop, 1969) it was pointed out that the effectiveness of amino acids in preventing lipid oxidation was greater when Cu^{++}, rather than Fe^{++}, was the catalyst. This again suggests that the mechanism of the inhibition is related to the affinity of the metal for complexing with the amino acid, which is greater for Cu^{++} than for Fe^{++}.

The differences in most of the reactions that have been observed between the lean and fatty fish might well be explained by the differences in the nature of their lipids, their abundance, and their physical distribution in the muscle. But this explanation will not hold for the difference in the reactions to added metals between lean fish and crustaceans or molluscs. The lipids of lobster muscle, for example, in terms of composition and concentration, are much closer to those of lean fish than to those of fatty fish. The lobster muscle, however, is very much less sensitive than lean fish muscle to metal-induced rancidity or changes in EPN. This also holds for all other types of shellfish and crustaceans that have been tested in this laboratory. It would appear that some other components in the fish muscle of these marine invertebrates complex with the metals, making them less available as lipid oxidation catalysts or for reacting with the proteins. It was shown previously (Castell and Spears, 1968) that the addition of an aqueous extract of lobster muscle to cod muscle protected the latter against Cu^{++}-induced rancidity. It is likely that there are many natural antioxidants in the muscle of various marine animals. One cannot help being struck by the fact, however, that these groups of marine invertebrates, which are naturally resistant to metal-induced changes in lipids and proteins, are also known to have relatively high concentrations of free amino acids. Although there are some variations among species, the muscles of marine crustaceans and molluscs contain in the order of 5–10 times as much free amino acids as the muscles of marine fish (Borgström, 1962).

Those concerned with changes that take place in stored frozen fish may ask what relation, if any, do these changes in extractable protein resulting from added metal ions have to texture changes that occur in improperly stored frozen fish? Cooking in itself will bring about more than 95% reduction in the

EPN in cod muscle, without producing the tough textured muscle characteristic of improperly stored frozen fish. Therefore, it is not simply a change in the solubility of the protein that produces toughness, even though the tough muscle invariably has greatly reduced EPN values. So far, there have been no observations to indicate that the rapid decrease of the EPN value of cod muscle, after the addition of small amounts of Cu^{++}, is accompanied by any change in the texture. Following the addition of linoleic or linolenic acid, and more specifically under conditions where these acids have undergone oxidation, marked changes in texture take place. If the metal ions that are normally found in fish do affect texture of frozen fish, our results suggest that it would most probably be through their action in oxidizing the unsaturated fatty acids in the muscle phospholipids; and that these in turn form insoluble polymers with the proteins.

REFERENCES

ALBERT, A. 1950. Quantitative studies of the avidity of naturally occurring substances for trace metals. 1. Amino acids having only 2 ionizing groups. Biochem. J. 47: 531–537.

BLIGH, E. G., AND W. J. DYER. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. 37: 911–917.

BORGSTRÖM, G. 1962. Shellfish protein — nutritive aspects. *In* G. Borgström [ed.] Fish as food, Vol. II. Academic Press, Inc., New York and London.

CASTELL, C. H., AND D. M. BISHOP. 1969. Effect of hematin compounds on the development of rancidity in muscle of cod, flounder, scallops, and lobster. J. Fish. Res. Bd. Canada 26: 2299–2309.

CASTELL, C. H., AND G. A. BOYCE. 1966. Erroneous thiobarbituric acid values in fish tissues caused by their normal content of free iron. J. Fish. Res. Bd. Canada 23: 1587–1598.

CASTELL, C. H., B. A. MOORE, AND W. E. NEAL. 1966. Cause and control of spurious thiobarbituric acid values from fish muscle in the presence of inorganic iron salts. J. Fish. Res. Bd. Canada 23: 737–746.

CASTELL, C. H., AND D. M. SPEARS. 1968. Heavy metal ions and the development of rancidity in blended fish muscle. J. Fish. Res. Bd. Canada 25: 639–656.

DYER, W. J., H. BROCKERHOFF, R. J. HOYLE, AND D. I. FRASER. 1964. Polyphosphate treatment of frozen cod. I. Protein extractability and lipid hydrolyses. J. Fish. Res. Bd. Canada 21: 101–106.

DYER, W. J., H. V. FRENCH, AND J. M. SNOW. 1950. Proteins in fish muscle. Extraction of protein fractions in fresh fish. J. Fish. Res. Bd. Canada 7: 585–593.

FRIEDEN, E. 1968. The biochemistry of copper. Sci. Amer. 218: 102–114.

FULLINGTON, J. G. 1967. Interaction of phospholipid — metal complexes with water-soluble wheat protein. J. Lipid Res. 18: 609–614.

FULLINGTON, J. G., AND H. S. HENDRICKSON. 1966. Interaction of triphosphoinositide and phosphatidyl serine-metal complexes with ethylenediamine, polyamino acids and protein. J. Biol. Chem. 241: 4098–4100.

LEA, C. H. 1936. Antioxidants and the preservation of edible fats. Dep. Sci. Ind. Res. (Brit.) Food Invest. Board 1935: 29–30.

1938. Rancidity in edible fats. Dep. Sci. Ind. Res. H. M. Stationery Office, London. 230 p.

1952. Rancidity in edible fats and fat-containing foods, p. 199–228. *In* E. C. Bate-Smith and T. N. Norris [ed.] Food science (Symposium). Cambridge University Press, London.

MARCUSE, R. 1967. The combined effect of amino acids and metals, p. 129–134. *In* R. Marcuse [ed.] Symposium on metal catalyzed lipid oxidation. S.I.K. Rapp. 1968, Nr. 240, Göteborg, Sweden.

ÖSTERBERG, R. 1967. Metal complexes of peptides, p. 119–127. *In* R. Marcuse [ed.] Symposium on metal catalyzed lipid oxidation. S.I.K. Rapp. 1968, Nr. 240. Göteborg, Sweden.

SNOW, J. M. 1950. Proteins in fish muscle. II. Colorimetric estimation of fish muscle protein. J. Fish. Res. Bd. Canada 7: 594–598.

TARLADGIS, B. G., B. M. WATTS, M. T. YOUNATHAN, AND N. DUGAN, JR. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Amer. Oil Chem. Soc. 37: 44–48.

Ultrasonic Tracking of Homing Cutthroat Trout (*Salmo clarki*) in Yellowstone Lake

JAMES D. MCCLEAVE

Department of Zoology
University of Maine, Orono, Maine, USA

AND

ROSS M. HORRALL

Laboratory of Limnology
University of Wisconsin, Madison, Wis., USA

MCCLEAVE, J. D., AND R. M. HORRALL. 1970. Ultrasonic tracking of homing cutthroat trout (*Salmo clarki*) in Yellowstone Lake. J. Fish. Res. Bd. Canada 27: 715–730.

Displacement of 42 mature cutthroat trout (*Salmo clarki*) from their spawning tributaries to the open water of Yellowstone Lake in the summers of 1966 and 1968 and following them ultrasonically up to 13.5 hr and 11.8 km indicated that those that homed used a compass mechanism rather than search or true navigation to find shore and then followed the shoreline to the home stream. Vision was unnecessary for homing. For many of the fish whose movements showed no consistent pattern, the transmitters failed prematurely.

Of 29 nonanesthetized trout tracked from a point northwest of their home streams, six moved generally eastward to shallow water, turned south, and followed the shoreline toward the home streams, three being followed all the way home. Six moved generally south or southeast and progressed toward their home streams entirely in open water, one being followed home. Three moved inappropriately for homing but were less than 2 km from the release point when tracking was ended. Fourteen moved randomly or did not move enough or were not tracked long enough to establish a pattern.

Of five anesthetized-blinded trout, two moved eastward and then south along the shoreline, one moved south in open water, and the other two did not show a consistent pattern. Of four anesthetized-control trout, two moved eastward and then south along shore, one being followed home; one moved inappropriately and the other did not establish a pattern.

Of four nonanesthetized trout tracked from a point southwest of their home stream, three moved east and then north along shore toward the home stream, one being followed home; the fourth moved north in open water.

Current directions may have influenced the initial orientations.

Swimming speeds ranged from 0.0 to 46.2 cm sec^{-1} in open water and from 8.5 to 82.3 cm sec^{-1} along shore. Average along-shore speed (36.6 cm sec^{-1}) was greater than average open-water speed (22.9 cm sec^{-1}). Blinded trout swam at about the same speeds (average 22.6 cm sec^{-1}) as control trout (average 27.0 cm sec^{-1}) and nonanesthetized trout (average 23.3 cm sec^{-1}).

Received April 8, 1969

Printed in Canada (J1435)

INTRODUCTION

MUCH USEFUL INFORMATION concerning the movements of fishes has been inferred from release–recapture studies and from short-term direction of takeoff studies. However, continuous "maps" of movements are necessary if orientation mechanisms are to be understood; what happens between release and recapture are the important features of migrations. This paper reports ultrasonic tracking experiments designed to provide such information for the homing migration of mature adfluvial cutthroat trout (*Salmo clarki*) displaced from their spawning tributaries to the open water of Yellowstone Lake, Yellowstone National Park, Wyoming.

Previously, in-season homing of control, blinded, and olfactory occluded trout displaced 0–22 km from their home streams into Yellowstone Lake was demonstrated (McCleave, 1967; Jahn, 1969). Displacement distance did not influence homing percentage or homing time. Blinding of trout caused no decrease in homing percentage, but it approximately doubled the homing time (as measured by recapture at the home stream trap). Results of olfactory occlusion were inconclusive.

Jahn (1966, 1969) and McCleave (1967) also reported general northeastward to southeastward orientation patterns of cutthroat trout in short-term float-tracking experiments designed to determine only direction of takeoff. Blinded and anosmic trout oriented just as well as control trout. Sun visibility, sun azimuth, and surface water currents were all implicated as influencing factors, but evidence was conflicting.

The ultrasonic tracking experiments were designed specifically to determine: (1) whether the homing movements of displaced trout were patterned over long distances; and if so (2) whether vision was necessary for the pattern to be maintained; (3) whether the pattern represented search, a compass orientation, or true navigation; and (4) what the actual speeds of migration were (as distinct from homing time to a fish trap). Some further information on influence of surface water currents was also obtained.

METHODS

All trackings except one were begun at either point B or point F in the northeast and east portions, respectively, of Yellowstone Lake, a large (354 km²), deep (98 m), high altitude (2358 m) lake (Fig. 1). The release points were selected in open water where shore and bottom features were least likely to influence the initial phases of trout movement. Both release points were located with the aid of a sextant and landmarks, and buoys positioned to mark them. From point B (depth about 40 m) Cub Creek was 134° and 4.0 km. Clear Creek was 146° and 5.3 km, and nearest land was 52° and 2.4 km. From point F (depth about 75 m) Clear Creek was 35°[1] and 5.2 km, and nearest land was 100° and 2.0 km. One tracking began 0.6 km south of point B.

In 1966, 21 nonanesthetized cutthroat trout were tracked beginning at point B, 16 of which were from Clear Creek and five from Cub Creek. In 1968, 17 trout were tracked beginning at point B, eight of which were nonanesthetized from Clear Creek, four anesthetized-control from Clear Creek, and five anesthetized-blinded from Clear Creek. In 1968 also, four nonanesthetized

[1]This bearing would allow a fish to miss a small jut of land and would require a short eastward correction near the end of the trip. Actual direction from point F to Clear Creek is 38°.

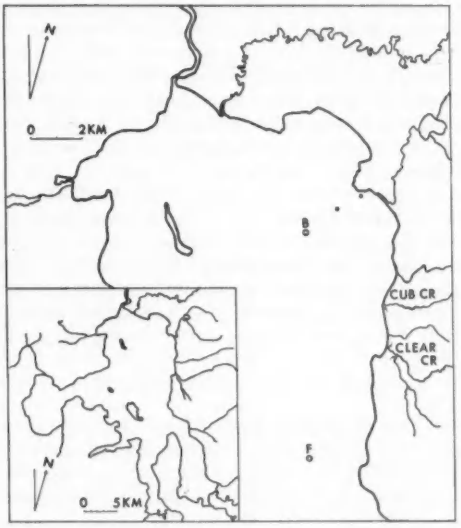


FIG. 1. Northern portion of Yellowstone Lake showing release points (B, F) and locations (arrows) where Clear and Cub creek trout were captured for tracking. Inset shows entire lake and principal tributaries.

Clear Creek trout were tracked from point F. Three tracks (5, 18, and 27) are not plotted, because the trout did not move significantly during the tracking period. On 12 occasions two trout were tracked simultaneously, allowing comparison of two trout under the same environmental and temporal conditions.

COLLECTION AND TRANSPORT OF TROUT

All trout tracked were sexually mature and were captured during their upstream migration to spawning grounds (Fig. 1). Most Clear Creek trout were captured in a fish trap located about 75 m above the creek mouth; one was captured with a dipnet, one by angling, and one in a fish trap on a small tributary that empties into Clear Creek about 0.7 km above its mouth. The Cub Creek trout were captured with a dipnet in the lower 200 m of the stream.

Usually 2–5 trout were captured and placed directly into a covered tub containing about 30 liters of water. The tub was then transported by boat to the release point — a 10–20-min trip depending on collection location and release point. Fresh water was added to the tub occasionally to prevent anoxia, and so the change from stream temperature to lake temperature was gradual. In one instance Clear Creek trout were transported 0.7 km in about 15 liters of water in a backpack to the mouth of Clear Creek for transfer to the boat.

If trout were to be anesthetized, the boat was stopped just offshore and the trout placed in a 1:7500 tricaine methanesulfonate (M.S. 222) solution. Trout were blinded by injecting 0.10–0.15 cm^3 of 3% aqueous benzethonium chloride (Phemerol) into the eyeball (McCleave, 1967). Control trout and blinded trout were returned to the covered tub of fresh water and allowed to recover from the anesthetic during the remainder of the trip to the release point.

TRACKING SYSTEM

The tracking system was essentially that described by Henderson et al. (1966). A National Radio Company LF-10 preselector was used in conjunction with a National HRO-500 receiver to increase sensitivity in the low frequency ranges. The directional hydrophone was a component of a Bendix DR-9 echo sounder.

The receiving equipment was installed in a 5-m Boston Whaler such that one person could operate the boat, receiver, and hydrophone from a seat at the console. In 1966 the hydrophone

was suspended 0.5 m below the surface off the bow by a vertical pipe mounted in pillow block bearings. A pulley system allowed the operator to rotate the horizontally directed hydrophone 360°. In 1968 the hydrophone was mounted off the starboard side near the console, the pulley system being eliminated. Also in 1968 the boat was provided with a canvas cover to protect equipment and operators against rain and waves breaking over the bow, allowing us to track under more adverse weather conditions than in 1966.

Part of the time two complete tracking boats were available so that two fish could be tracked simultaneously.

The ultrasonic tags (transmitters) have been described by Henderson et al. (1966). Values of the circuit components had to be determined separately for each different lot of the lead zirconium titanate transducers in order for them to oscillate properly under water and with a minimum of current drain. Frequencies ranged from 30 to 75 kHz. A miniature alligator clip was glued to the battery end of the transmitter with Duco cement, and the transmitter clipped to the posterior edge of the trout's dorsal fin (McCleave et al., 1967). The transmitter itself was a cylinder about 9 × 40 mm; addition of the alligator clip increased the length to about 60 mm. Stomach placement of the tag, successful with white bass (Henderson et al., 1966; Hasler et al., 1969), was attempted, but this sometimes resulted in rupture of the esophagus in cutthroat trout, and so was not used.

The transmitters were constructed and waterproofed in advance, the negative battery connection being left open. In 1966 the tags were activated before leaving our living quarters on tracking days by soldering the final connection and dipping in a 1:1 mixture of Duco cement and *n*-butyl acetate, or were activated in the field by twisting the two wires together and dipping. In 1968 all tags were activated in the field just prior to use with a propane torch soldering tip or a 12-v d-c soldering iron. Waterproofing was accomplished by dipping or with a coating of silicone grease.

Pretracking Procedure

In 1966 upon arrival at the release point we first assembled the receiving gear and calibrated the HRO-500 with its internal crystal-controlled calibration signal. This feature allowed us to read the frequency of the ultrasonic tag directly in kHz from the tuning dial of the receiver. Next, the tag, which had been activated earlier, was attached to the thermistor probe of a Whitney TC-5 underwater thermometer and lowered into the water. The water temperature and tag frequency were recorded at depths of 1, 5, 10, 15, and 20 m. This assured us that the tag was operating properly under pressure in water, and also gave its frequency response to temperature and pressure.[2] If a tag failed during this test, a spare was activated by twisting the leads and water proofing. The tag was then clipped to the trout, and the trout released immediately by hand, placed back in the tub, or placed in a release net. If it was placed in the tub, the tub was lowered over the side of the boat and the trout allowed to swim out. If the fish was placed in a release net,

---

[2]Preliminary laboratory tests of transmitters in a pressure chamber had shown that the frequency was independent of pressure to a simulated depth of 30 m. This was verified in the field on two occasions, when tags lowered to 20 m under isothermal conditions showed no frequency change. Eliminating depth, then, a linear regression of temperature on frequency was calculated from the field data on 54 transmitters, giving the equation

$$Y = F + 0.409 - 0.087T$$

where Y = frequency (kHz) at temperature at selected depth; F = frequency (kHz) at temperature at 1 m depth; and T = change in temperature (C) from 1 m to selected depth. Therefore, for an increase in temperature of 1 C, a decrease in frequency of about 90 Hz is expected, and vice versa. Analysis of variance showed the regression to be significant at $P = 0.005$. However, under usual temperature versus depth conditions in Yellowstone Lake this relation allowed us only to determine whether the trout being tracked was above, in, or below the metalimnion.

an approximately 1 × 2-m cylindrical hoop net suspended from the buoy, the tracking boat usually moved away to get an estimate of transmitter range. Then the bottom of the release net was opened, and the trout allowed to swim out.

The same general procedure was followed in 1968, but all tags were activated at the release point, and for the last 16 trackings a 1-m drift drogue (Jahn, 1966) was released immediately upon arrival at the release point. After testing, the tag was attached to the buoy and the tracking boat moved away to estimate range. A sextant sighting using landmarks, or a compass bearing, was then taken on the drogue, and it was retrieved. The boat returned to the buoy where the trout was tagged and released, initiating the tracking.

Departures from the above procedure were made when we felt this would expedite the operation. For example, if a tag failed during a depth test, we often omitted a depth test on the spare tag in order not to delay the beginning of a track. Or if weather were threatening, some steps were eliminated to speed the pretracking procedure. When simultaneous trackings were to be made, one of us often went directly to the release point to begin testing equipment, while the other collected and transported the trout.

Tracking Procedure

After trout were released the boat remained near the release point until the signal from the tag became weak, and then was moved closer on a bearing obtained from the hydrophone. Trout were followed by alternately waiting and moving up. The frequency of this maneuver depended upon speed of trout movement, range of tag, and weather conditions. The strength of the received signal was not a reliable indicator of range, but in general a weak, highly directional signal came from a distant tag, and as the boat moved closer the signal became stronger and less directional. Directly over the trout the signal was omnidirectional and strong.

The trout's position was plotted whenever it had markedly changed location or direction, rather than on a fixed time schedule. The time interval between plots varied from a few minutes to over an hour. The first position plot was occasionally several hours after release, if the trout was slow to move.

Position plots in open water were obtained by measuring the two adjacent horizontal angles between three landmarks with a U.S. Navy Mark II type sextant. Poor light conditions or high waves decreased the accuracy of some plots. Position plots along shore were usually determined by observing on a topographic map the land features being passed. Some sextant sightings were also made.

Although no alteration in behavior was detected when the boat moved over the fish, we usually remained a short distance away from the trout while a position plot was being made. This was less accurate, but decreased the possibility of disturbing the fish.

The trackings were terminated for the following reasons: 28 when the tag ceased to operate, 3 due to bad weather, 5 when the trout had returned to the home stream, 3 due to operator fatigue, 2 due to darkness, and 1 due to engine trouble. None was terminated because the trout exhibited movement we considered inappropriate.

Throughout each tracking, observations were made on sun visibility, cloud cover, precipitation, wind speed, wind direction, and water surface conditions. No changes in movement behavior were detected that could be associated with any of these environmental factors. However, no long periods of overcast skies occurred while fish were being tracked. For some fish, a depth sounder was used to record water depth at position plots as the trout approached or followed the shoreline.

Plotting and Measurement of Tracks

Each track was plotted on a large-scale work map of Yellowstone Lake. A three-arm protractor was used to locate the sextant sightings, and field notes to locate other position plots along shore. The open-water swimming distance and along-shore swimming distance were measured from each map. Open-water, along-shore, and total swimming speeds for each trout were then calculated.

For the final 16 trackings the current direction (degrees from true North) immediately before tracking was subtracted from the direction (degrees from true North) of the trout at the first available hourly observation, and from the direction when the trout reached a distance of 0.5 km from the release point. These converted data were used to calculate mean vectors (mean direction and vector length) (Batschelet, 1965) based on water current direction as the zero direction for comparison with mean vectors based on true North (McCleave, 1967). Rayleigh's test (Batschelet, 1965) was used to determine if the vector length was significantly greater than zero.

MOVEMENT PATTERNS

Movements of the 29 nonanesthetized Clear and Cub Creek trout tracked at point B were distinctly patterned. Each track was assigned (somewhat arbitrarily) to one of four categories depending on the movement exhibited (Fig. 2, Tables 1, 2).

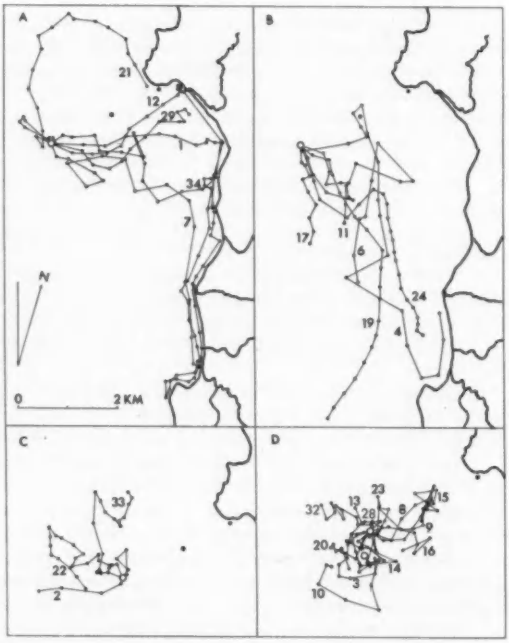


FIG. 2. Tracks of 27 nonanesthetized Clear and Cub creek trout beginning at point B (tracks of two fish not plotted). Small circles indicate position plots. (A) 6 trout in eastward-moving category; (B) 6 trout in southeastward-moving category; (C) 3 trout in inappropriately moving category; (D) 12 trout in random or undetermined category (one released at the second site).

(1) Six trout from Clear Creek went generally eastward until they reached shallow water, then turned south, and followed the shoreline toward their home stream (Fig. 2A). They were tracked an average of 8 hr 29 min and 7.8 km. Three of the fish (tracks 7, 12, 34) were followed all the way back to the mouth of Clear Creek. During the open-water portion of the tracks some of the trout moved long distances in nearly straight lines, whereas others changed directions several times. Upon reaching shallow water four of the

TABLE 1. Ultrasonic trackings of cutthroat trout in 1966 and 1968. Clear Creek trout captured in fish trap unless otherwise noted; Cub Creek trout captured by dipnet. Track numbers in parentheses indicate simultaneous trackings.

Track no.	Capture location	Release date	Tracking time (MST)	Recapture location	Recapture date
		Nonanesthetized trout, Point B			
1	Clear Cr.	14-VII-66	1010–1300		
2(3)	" "	15-VII-66	0813–1141		
3(2)	" "	15-VII-66	0958–1158	Clear Cr.	16-VII-66
4(5)	" "	20-VII-66	0730–1400	Cub Cr.	23-VII-66
5(4)	" "	20-VII-66	0730–1216		
6(7)	" "	21-VII-66	0735–1153	Clear Cr.	26-VII-66
7(6)	" "	21-VII-66	0748–1525	" " [a]	3-VIII-66
8	" "	22-VII-66	0757–1320	" "	30-VII-66
9(10)	" "	23-VII-66	0725–1115	" "	25-VII-66
10(9)[b]	" "	23-VII-66	0815–1135	" " [a]	9-VIII-66
11(12)	" "	24-VII-66	0744–1120		
12(11)	" "	24-VII-66	0744–1644		
13(14)	" "	26-VII-66	0732–1307	" "	1-VIII-66
14(13)	" "	26-VII-66	0734–0919		
15	Cub Cr.	28-VII-66	0905–1330		
16(17)	" "	29-VII-66	0821–1112		
17(16)	" "	29-VII-66	0821–1155	Cub Cr. [a]	1-VIII-66
18(19)	" "	30-VII-66	0826–1040		
19(18)	" "	30-VII-66	0826–1627		
20	Clear Cr.[c]	1-VIII-66	0824–1324		
21	" " [c]	2-VIII-66	0846–1713		
22	" " [d]	25-VI-68	0828–1258		
23	" "	26-VI-68	0735–1120		
24	" " [e]	1-VII-68	1335–2105		
28(29)	" "	6-VII-68	1051–1148		
29(28)	" "	6-VII-68	1140–2110		
32(33)	" "	9-VII-68	0625–1125		
33(32)	" "	9-VII-68	0625–1225		
34	" "	12-VII-68	0650–2020		
		Blinded trout, Point B			
25	" "	4-VII-68	0934–1538		
27(26)	" "	5-VII-68	0900–1608	Clear Cr.	22-VII-68
31(30)	" "	7-VII-68	1132–1700		
35	" "	14-VII-68	0625–1822	Clear Cr.	15-VII-68
37	" "	18-VII-68	0620–1230		
		Anesthetized control trout, Point B			
26(27)	" "	5-VII-68	0900–1800		
30(31)	" "	7-VII-68	1132–1300		
36	" "	16-VII-68	0630–1542		
38	" "	21-VII-68	0630–1350	Clear Cr.	22-VII-68
		Nonanesthetized trout, Point F			
39	" "	22-VII-68	0710–1710	Clear Cr.[a]	24-VII-68
40	" "	24-VII-68	0645–1213		
41	" "	25-VII-68	0650–1659		
42	" "	27-VII-68	0630–1500		

[a] Ultrasonic tag found in fish trap, not on fish.
[b] Released about 0.6 km south of Point B.
[c] Captured with dipnet.
[d] Trapped in small tributary entering Clear Creek about 0.7 km above Clear Creek mouth.
[e] Captured by angling.

TABLE 2. Tracking times, minimum swimming distances, and minimum swimming speeds of cutthroat trout during open-water portions, along-shore portions, and entire tracks. Figures in parentheses eliminate periods of little or no movement that occurred at the beginning or end of some tracks, giving better estimates of actual swimming speed.

Track no.	Open-water portion			Along-shore portion			Entire track		
	Distance (*km*)	Time (*hr:min*)	Speed (*cm sec⁻¹*)	Distance (*km*)	Time (*hr:min*)	Speed (*cm sec⁻¹*)	Distance (*km*)	Time (*hr:min*)	Speed (*cm sec⁻¹*)
				Nonanesthetized trout, Point B					
1	3.43	2:23	40.0	1.33	0:27	82.3	4.76	2:50	46.7
2	3.22	3:28	25.8				3.22	3:28	25.8
3	2.39	2:00	33.2				2.39	2:00	33.2
	(2.01)	(1:00)	(55.8)				(2.01)	(1:00)	(55.8)
4	9.18	6:00	42.5	1.25	0:30	69.4	10.43	6:30	44.6
	(9.08)	(5:30)	(45.9)				(10.33)	(6:00)	(47.8)
5	0.00	4:46	00.0				0.00	4:46	00.0
6	7.15	4:18	46.2				7.15	4:18	46.2
	(6.67)	(3:17)	(56.4)				(6.67)	(3:17)	(56.4)
7	7.46	6:00	34.5	1.57	1:37	26.9	9.02	7:37	32.9
8	3.52	5:23	18.2				3.52	5:23	18.2
	(3.04)	(3:35)	(23.6)				(3.04)	(3:35)	(23.6)
9	2.84	3:04	25.7				2.84	3:04	25.7
10	3.22	3:20	26.9				3.22	3:20	26.9
	(2.89)	(2:33)	(31.5)				(2.89)	(2:33)	(31.5)
11	2.77	3:36	21.4				2.77	3:36	21.4
12	3.22	2:33	35.0	8.57	6:27	36.9	11.78	9:00	36.4
	(3.08)	(2:24)	(35.7)	(8.27)	(5:15)	(43.7)	(11.35)	(7:39)	(41.2)
13	2.89	5:35	14.4				2.89	5:35	14.4
14	1.88	1:45	29.9				1.88	1:45	29.9
	(1.57)	(0:50)	(52.2)				(1.57)	(0:50)	(52.2)
15	4.08	4:25	25.6				4.08	4:25	25.6
	(4.01)	(4:05)	(27.3)				(4.01)	(4:05)	(27.3)
16	2.43	2:51	23.7				2.43	2:51	23.7
	(2.36)	(2:22)	(27.7)				(2.36)	(2:22)	(27.7)
17	2.27	3:34	17.6				2.27	3:34	17.6
18	0.00	2:14	00.0				0.00	2:14	00.0
19	6.45	8:01	22.4				6.45	8:01	22.4
20	2.01	5:00	11.2				2.01	5:00	11.2
	(1.84)	(2:39)	(19.3)				(1.84)	(2:39)	(19.3)
21	5.72	8:00	19.9	0.40	0:27	24.7	6.12	8:27	20.1
22	4.48	4:30	27.7				4.48	4:30	27.7
23	2.05	3:45	15.2				2.05	4:35	15.2
	(1.50)	(2:08)	(19.5)				(1.50)	(2:08)	(19.5)
24	6.15	7:30	22.7				6.15	7:30	22.7
	(5.95)	(6:10)	(26.8)				(5.95)	(6:10)	(26.8)
28	0.60	0:57	17.5				0.60	0:57	17.5
29	5.12	8:34	16.6	0.28	0:56	8.5	5.40	9:30	15.8
	(4.42)	(5:46)	(21.3)	(0.28)	(0:21)	(22.5)	(4.70)	(6:07)	(21.3)
32	2.70	5:00	15.0				2.70	5:00	15.0
33	3.92	6:00	18.1				3.92	6:00	18.1
34	5.38	8:55	16.8	4.23	4:35	25.7	9.62	13:30	19.8
	(5.10)	(3:55)	(36.2)				(9.33)	(8:30)	(30.5)
Mean	3.71	4:36	22.9	2.52	2:08	39.2	4.32	5:07	23.3
	(3.78)	(3:56)	(28.9)	(2.48)	(1:53)	(42.2)	(4.42)	(4:26)	(29.1)
				Blinded trout, Point B					
25	6.47	6:04	29.6				6.47	6:04	29.6
	(5.65)	(5:02)	(31.2)				(5.65)	(5:02)	(31.2)
27	0.00	7:08	00.0				0.00	7:08	00.0
31	4.48	5:28	22.8				4.48	5:28	22.8

(*Continued*)

TABLE 2. Tracking times, minimum swimming distances, and minimum swimming speeds of cutthroat trout during open-water portions, along-shore portions, and entire tracks. Figures in parentheses eliminate periods of little or no movement that occurred at the beginning or end of some tracks, giving better estimates of actual swimming speed. (*Concluded*)

	Open-water portion			Along-shore portion			Entire track		
Track no.	Distance (*km*)	Time (*hr:min*)	Speed (*cm sec⁻¹*)	Distance (*km*)	Time (*hr:min*)	Speed (*cm sec⁻¹*)	Distance (*km*)	Time (*hr:min*)	Speed (*cm sec⁻¹*)
35	6.87	9:16	20.6	3.48	2:41	36.1	10.35	11:57	24.1
	(6.70)	(7:24)	(25.2)				(10.18)	(10:05)	(28.1)
37	8.05	6:10	36.3				8.05	6:10	36.3
	(7.72)	(4:57)	(43.3)				(7.72)	(4:57)	(43.3)
Mean	5.17	6:49	21.9	3.48	2:41	36.1	5.87	7:21	22.6
	(6.14)	(5:43)	(30.6)				(7.01)	(6:23)	(31.4)
Anesthetized control trout, Point B									
26	9.72	9:00	30.0				9.72	9:00	30.0
	(9.67)	(8:30)	(31.6)				(9.67)	(8:30)	(31.6)
30	0.53	1:28	10.1				0.53	1:28	10.1
	(0.42)	(0:58)	(12.0)				(0.42)	(0:58)	(12.0)
36	5.45	6:38	22.8	2.27	2:34	24.5	7.72	9:12	23.3
	(4.85)	(4:23)	(30.7)				(7.12)	(6:57)	(28.5)
38	3.60	2:25	41.4	8.12	4:55	45.9	11.71	7:20	44.4
				(7.98)	(4:05)	(54.2)	(11.57)	(6:30)	(49.4)
Mean	4.83	4:53	26.1	5.20	3:45	35.2	7.42	6:45	27.0
	(4.64)	(4:04)	(28.9)	(5.13)	(3:20)	(39.4)	(7.20)	(5:44)	(30.4)
Nonanesthetized trout, Point F									
39	3.72	7:57	13.0	1.93	2:03	26.2	5.65	10:00	15.7
	(3.48)	(4:12)	(23.0)				(5.42)	(6:15)	(24.1)
40	4.87	4:16	31.7	1.63	1:12	37.8	6.50	5:28	33.0
41	3.73	4:20	23.9	6.50	5:49	31.0	10.23	10:09	28.0
				(5.55)	(4:34)	(33.8)	(9.28)	(8:54)	(29.0)
42	5.30	8:30	17.3				5.30	8:30	17.3
	(4.30)	(6:12)	(19.3)				(4.30)	(6:12)	(19.3)
Mean	4.41	6:16	21.5	3.35	3:01	31.7	6.92	8:32	23.5
	(4.10)	(4:45)	(24.5)	(3.04)	(2:36)	(32.6)	(6.37)	(6:42)	(26.4)
Grand mean	4.06	5:03	22.9	3.20	2:38	36.6	5.04	5:52	23.6
	(4.15)	(4:13)	(28.6)	(3.09)	(2:20)	(42.3)	(5.16)	(5:00)	(29.2)

trout immediately turned south. Two turned north for a short distance, then reversed direction, and moved southward. The along-shore movement was usually less than 100 m from shore, and in water less than 10 m deep. Sometimes they were less than 15 m offshore in water less than 5 m deep. In some instances trout appeared to follow irregularities in the shoreline, but in others they apparently cut across bays in straight lines. Little, if any, delay occurred when the trout passed stream mouths, although a short hesitation might not be detected by this method. When the mouth of the home stream was reached, however, the trout swam back and forth in the vicinity. One trout (track 12) hesitated at the mouth of Clear Creek, continued south along shore for more than 1 km, then returned to the stream mouth.

(2) Six trout, four from Clear Creek and two from Cub Creek, moved generally southward or southeastward, making progress toward the home stream entirely in open water (Fig. 2B). They were tracked an average of 5 hr 35 min and 5.9 km. Movements in open water were variable among this group also. Some trout moved in straight paths; others changed directions often. One (track 4) was followed to the mouth of its home stream, Clear Creek, which it passed, and moved toward Cub Creek, where it was recaptured. Another (track 24) nearly reached the home stream when it became dark; however, the trout apparently stopped moving at that time. One trout (track 19) went too far south in open water for direct homing. The other three moved lesser distances.

(3) Three trout, tracked an average of 4 hr 42 min and 3.9 km, made movements we considered inappropriate for direct homing (Fig. 2C). One moved north, one west, and one northwest; however, the last had reversed its direction, moved southeast, and was less than 1 km from the release point at the termination of the track. None moved more than 2 km in an inappropriate direction.

(4) The remaining 14 trout, tracked an average of only 3 hr 35 min and 2.2 km, moved randomly, did not move enough to establish a pattern, or were not tracked long enough to determine a pattern (Fig. 2D). Five of these moved generally eastward, three of them to the vicinity of a small island about 1.5 km northeast of point B.

Despite the variability in the trout movements these tracks demonstrated that cutthroat trout were able to travel distances of at least 3–4 km in fairly straight paths, and to home in as little as 6–8 hr either by moving eastward and following the shoreline or, more directly, by moving southeastward in open water. Many trout in the last two categories may have moved eastward or southeastward after the trackings were terminated, for 5 of the 14 trout in the undetermined category were later recaptured in the home stream. These recaptures indicate that rapid initial orientation toward the home stream is not necessary for homing.

Vision was not necessary for the homing movement patterns since the patterns exhibited by the five anesthetized-blinded trout (Fig. 3A) were similar to those of the four anesthetized-control trout (Fig. 3B) and the 29 nonanesthetized trout (Fig. 2). The blinded trout were tracked an average of 7 hr 21 min and 5.9 km, and the controls 6 hr 45 min and 7.4 km. Two of the blind trout moved eastward, then south along the shoreline, and another blind trout made considerable progress southward in open water. The latter (track 37) moved more than 2 km in a nearly straight path. The other two blind trout were in the undetermined category. Two of the control trout also moved eastward to shore: one turned south immediately; the other (track 38) moved northward along shore over 1 km, then reversed direction and followed the shoreline south to the mouth of Clear Creek, passed it slightly, and then returned to it. Shortly afterward the tagged trout was sighted about 10 m inside the stream mouth, and a few minutes later was observed just below

the fish trap. It was recaptured in the trap the following day. One control trout (track 26) made the longest inappropriate movement observed. It went westward to the vicinity of Stevenson Island, then reversed direction, and was headed eastward toward the release point when the tracking was terminated. The fourth control trout was not tracked long enough for much movement to occur.

Two of the blinded and one of the control trout (mentioned above) were recaptured in Clear Creek. One of the blinded trout (track 27) did not move from the release point in the 7 hr it was tracked. It was recaptured 17 days later. However, another blinded trout (track 35) was well on its way toward home following the shoreline south after 12 hr. It was recaptured the day after tracking.

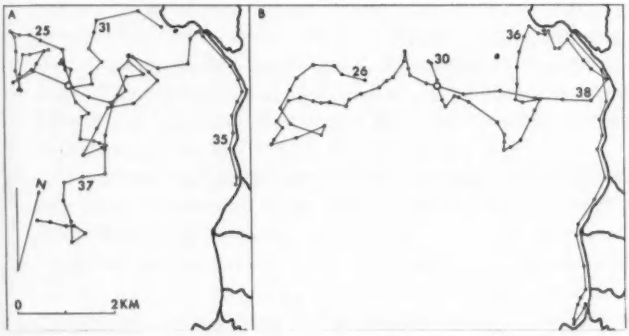


FIG. 3. Tracks of (A) four anesthetized-blinded Clear Creek trout from point B (track of one fish not plotted); (B) four anesthetized-control Clear Creek trout from point B.

FIG. 4. Tracks of four nonanesthetized Clear Creek trout from point F.

The evidence we compiled, although much of it was indirect, suggested that the cutthroat trout home from open water by means of a compass orientation (type II: Griffin, 1952; Hasler et al., 1958) rather than by search and piloting (type I) or true navigation (type III).

The most direct evidence came from comparison of the movement patterns of four nonanesthetized trout from Clear Creek tracked from point F (Fig. 4), a southerly release point, with the movement patterns of trout tracked from point B (Fig. 2, 3), a northerly release point. The point F fish were tracked an average of 8 hr 32 min and 6.9 km. Three of these trout moved generally eastward until they reached shallow water, where they immediately turned north and followed the shoreline toward the home stream. One of these (track 41) was followed the entire distance to the Clear Creek mouth, where it was seen, and another (track 39) was recaptured in the Clear Creek trap 2 days after tracking. These tracks show that there was an appropriate change in the eastward movement pattern between the north and south release points: the direction chosen at the shoreline was always appropriate to take the trout

to the home stream. The fourth trout (track 42) moved northward in open water, but then changed directions and stopped moving.

On the basis of this comparison true navigation cannot be ruled out, but it seems unlikely since the predominant movement pattern both at point B and at point F was eastward and then along shore. If trout were navigating by retracing a displacement route, they should have moved in a nearly straight path home, since the transportation from the home stream to the release point was more or less in a straight line. If trout were detecting differences in some set of physical coordinates between the stream location and the release point location, the homing navigation need not be along a straight path. However, a general pathway in the homeward direction would be predicted, rather than some other consistent pattern. A southeastward movement direction would have been expected from trout at point B, and a northeastward movement from trout at point F. A few trout did move southeastward from point B, but this was not the predominant pattern.

The eastward or southeastward open-water orientation of homing cutthroat trout is not explained by random or patterned search and piloting, although piloting may well be used in later portions of the homing movements. It is possible that the trout in the course of their life history have travelled over much of the lake, since the populations from the spawning tributaries do intermingle in the lake (Cope, 1957). However, in open water any features of or in the water itself are continually moved by the currents, and could not be used as fixed reference points in the sense of familiar landmarks. Release points B and F were selected in deep water (40 and 75 m) to prevent use of bottom features for orientation. Except for an initial escape dive by some, trout swam in the epilimnion, the upper 10–15 m, after stratification occurred. We determined this by noting frequency changes of the transmitters and by the directional characteristics of the received signal. Jahn (1969) has taken underwater photographs from a midlake point toward shore at depths of 0.5, 1.0, and 2.0 m in Yellowstone Lake. The altitude angles above the shoreline of prominent shore features (mostly mountain tops) were about the same from his photographic station as from our release points (3–4°). No shore features were visible in the photographs.

The movements of some trout in open water may have been random. However, most of those followed for a considerable distance tended to move in a general direction, and this movement did not resemble a search pattern.

SWIMMING SPEEDS

Trout swam faster during the along-shore portions of the tracks than during the open-water portions. Swimming speeds ranged from 0.0 to 46.2 cm sec^{-1} in open water and from 8.5 to 82.3 cm sec^{-1} along shore (Table 2). The average along-shore speed (36.6 cm sec^{-1}) was greater than the average open-water speed (22.9 cm sec^{-1}) ($t = 3.22$ with 53 df, $P < 0.01$). All but 2 of the 13 trout having shore components to their movements were slower in open water than along shore.

Blinded trout not only exhibited the same movement patterns as control trout and nonanesthetized trout but also swam at about the same speeds. Blinded trout swimming speeds (average 22.6 cm sec^{-1}) were not significantly different from those of control trout (average 27.0 cm sec^{-1}) ($t = 0.47$ with 7 df), or from those of nonanesthetized trout tracked at point B (average 23.3 cm sec^{-1}) ($t = 0.12$ with 32 df).

Swimming speeds of the six trout tracked all the way to their home stream were 44.6, 44.4, 36.4, 32.9, 28.0, and 19.8 cm sec^{-1} (tracks 4, 38, 12, 7, 41, 34, respectively). Their average swimming distance was 10.49 km, so in homing they swam about twice the straight line distance from the release point to the home stream (5.3 km). All six had along-shore components to their movements, but one (track 4) essentially homed in open water.

Swimming speeds expressed as fish total lengths per second were calculated for the seven trout whose lengths were measured upon recapture. The open-water speeds were 1.30, 1.12, 0.92, 0.65, 0.47, 0.38, and 0.00 L (length) sec^{-1} (tracks 6, 38, 3, 9, 8, 13, 27, respectively). One (track 38) also had a shore component of 1.24 L sec^{-1}. These swimming speeds were as high as 1.58 L sec^{-1}, if only the time during the tracking in which movement actually occurred is considered.

All reported distances are minimum estimates, since straight paths were assumed between position plots. Therefore, all swimming speeds are also minimum estimates.

WATER CURRENTS

Water currents in the upper few meters probably influenced the initial orientation of the trout, and this may explain why an initial northwestward or northward orientation often occurred and gradually shifted toward the east (e.g., Fig. 4). For the 16 tracks in which measurements were made the 1-m current directions were highly clustered to the west-northwest for all groups of tracks except the controls, which showed some concentration of currents to the northeast (Table 3). The vector length of the combined current directions was greater than zero ($P < 0.01$). The combined uncorrected trout vectors were not significantly greater than zero either at the hour observation or the 0.5 km observation. However, the corrected vector lengths were higher than the uncorrected ones for all groups of trout except the nonanesthetized point B group, and the combined track vector lengths were greater than zero ($P < 0.01$). The corrected mean directions were close to the current directions (0° or 360°). Trout affected most by water currents were apparently those that had been anesthetized prior to tracking.

GENERAL DISCUSSION

Direction of takeoff data from short-term float-tracking experiments in Yellowstone Lake have consistently shown that cutthroat trout from Clear, Cub, and Pelican creeks orient generally eastward when displaced to open water (Jahn, 1966, 1969; McCleave, 1967). By extending experiments on

TABLE 3. Mean directions and vector lengths at first available hourly and first 0.5-km observations of 16 Clear Creek trout tracked in 1968 based on true North (uncorrected) and current direction (corrected) as the zero directions. Current measurements made with 1-m drogue just prior to the start of a track.

Exptl. group	Release point	No. tracked	Current drogue		Trout, uncorrected		Trout, corrected	
			Mean direction (*a*)	Vector length (*r*)	Mean direction (*a*)	Vector length (*r*)	Mean direction (*a*)	Vector length (*r*)
First hourly observation								
Blinded, anesthetized	B	4	289°	0.8365	34°	0.0678	285°	0.3273
Control, anesthetized	B	4	41	0.3599	9	0.1067	1	0.9295
Nonanesthetized	B	4	285	0.9613	336	0.7544	37	0.5171
Nonanesthetized	F	4	306	0.8676	300	0.8690	354	0.9786
Combined	–	16	301	0.6356[a]	322	0.4080[b]	358	0.6007[c]
First 0.5-km observation								
Blinded, anesthetized	B	4	289	0.8365	27	0.0697	297	0.3295
Control, anesthetized	B	4	41	0.3599	37	0.3462	20	0.9144
Nonanesthetized	B	4	285	0.9613	342	0.5926	17	0.3403
Nonanesthetized	F	4	306	0.8676	304	0.8884	358	0.9804
Combined	–	16	301	0.6356[a]	333	0.3756[d]	1	0.5964[e]

[a]Rayleigh test: $z = 6.46$; significant at $P = 0.01$. Individual group samples too small for test.
[b]Rayleigh test: $z = 2.66$; nonsignificant.
[c]Rayleigh test: $z = 5.77$; significant at $P = 0.01$.
[d]Rayleigh test: $z = 2.26$; nonsignificant.
[e]Rayleigh test: $z = 5.69$; significant at $P = 0.01$.

direction of takeoff to longer-term ultrasonic trackings of Clear and Cub creek trout we have substantiated the hypothesis that the eastward takeoff orientation represented the initial part of an eastward or southeastward homing movement pattern, at least in those two populations.

Pelican Creek is on the north shore, and Clear and Cub creeks on the east shore, but all three are actually in the northeast portion of the lake. Whether the eastward movement is characteristic of all spawning populations of cutthroat trout in Yellowstone Lake, or just those from the eastern portion, is as yet unknown. Jahn (1969) float-tracked 23 trout from Arnica Creek in the western "thumb" of Yellowstone Lake. Their mean direction from a mid-lake release point was also eastward, but the vector length was not significant.

The question of how general the eastward movement pattern is could be resolved by ultrasonic tracking of trout from the western portion of the lake. However, these spawning runs are early (late May–early June), and the trout have to be displaced from relatively warm stream water into lake water at or below 4 C. Bad weather makes tracking difficult at that time.

Probably a general eastward compass mechanism would serve to direct the trout quickly to near-shore areas, where many of their normal activities occur. Ultrasonic tracking in Lake Mendota has recently demonstrated that both spawning and postspawning white bass (*Roccus chrysops*) have a compass mechanism to bring them to shallow areas in the eastern portion of the lake (Hasler et al., 1969). No nonspawning cutthroat trout were tracked, but they

do feed extensively along shore. The homing movement pattern exhibited by the trout may be an extension of a more general movement pattern exhibited by any fish finding itself in open water as Hasler et al. (1969) suggest.

Any compass orientation that would allow a trout to find shore quickly would be an advantage over random movement even if the trout found shore on the opposite side of the lake from the home stream. Yellowstone Lake has a shoreline of about 200 km, which at 30 cm sec^{-1} swimming speed could be covered in about 185 hr. So if a trout from one side of the lake encountered shore on the wrong side, it could follow the shoreline home in about 90 hr. If the compass orientation directed the trout to the appropriate shore, the search time would be reduced tremendously.

The fact that the cutthroat trout at the shoreline always turned in the appropriate direction, or at least corrected their direction after only a short error, represents a still further increase in the efficiency of homing. Once trout encountered the shoreline they did not return to open water. It is interesting to note that several trout apparently mistook a small island for the shore, at least temporarily (tracks 4, 8, 9, 15 in Fig. 2; 35, 37 in Fig. 3A).

Once the decision is made to follow the shoreline in a given direction no further compass orientation is necessary. The trout can simply swim along the shoreline until they somehow recognize their home stream. This may be why trout swam faster along shore than in open water, where continual orientation is necessary to maintain the chosen direction. The slower open water swimming could also have been due to periods of inactivity immediately after release. However, a consideration of only the movement portions of the tracks (Table 2) suggests that this was probably not the case. It is also possible that the tracking and plotting procedure minimized the open-water swimming distance more than the along-shore swimming distance.

The sensory mechanism(s) by which the compass operates remains elusive. Exhibition of the same pattern by blind and control trout indicates that vision is not necessary, but doesn't preclude its use by normal trout. This is in agreement with earlier tagging and float-tracking experiments in which blinded trout oriented and homed just as well as control trout (McCleave, 1967; Jahn, 1969). The slow homing times reported for blinded trout in those studies were probably due to delay in entering the stream or fish trap, not the open-water journey, since migration speeds of blinded trout in this study were comparable to speeds of control and nonanesthetized trout. Jahn (1966) observed some disorientation of float-tracked trout under overcast skies, but sample sizes were small. Anosmic trout oriented just as well as controls when float-tracked (McCleave, 1967), but their homing performance is in doubt due to faulty occlusion techniques employed. No anosmic trout were tracked with ultrasonic techniques.

The means by which trout are able to make the correct direction choice at the shoreline are not known either. It is possible that they are familiar with the shoreline features, at least on the home stream side of the lake; however, blind trout are also able to make the correct choice of direction. Correct choices

were made under surface conditions ranging from flat calm to more than 1-m waves, so wave patterns, wave sounds, and currents were probably not providing any directional cues. These surface conditions are also probably the same along shore both north and south of Clear and Cub creeks. Home stream odor may influence this choice, since Clear and Cub creek waters flow in a northerly direction along shore for some distance at least.

In waters similar in size to Yellowstone Lake, or even much larger, a simple compass orientation to bring a fish to shore (where a two-dimensional orientation problem becomes a one-dimensional one) on the same side of the lake as the home stream, and a means for choosing the direction along shore that leads to the home stream, would provide a great increase in homing efficiency over search and piloting. Yet it requires no postulation of a complex navigation ability. This seems to be the mechanism of homing by cutthroat trout in Yellowstone Lake.

ACKNOWLEDGMENTS

We thank Drs C. J. D. Brown, L. A. Jahn, G. W. LaBar, and Q. J. Stober for their help in various aspects of this study, and Dr W. H. Sippel for generously loaning a house trailer for our use. The cooperation of the U.S. Fish and Wildlife Service and the U.S. National Park Service is appreciated. Funds were supplied by the National Science Foundation (grant no. GB-3512 to Dr C. J. D. Brown, grant no. GB-7646 to Brown and McCleave) and the Office of Naval Research (contract nonr-4840 to Brown). Support was also provided by the National Science Foundation (grants no. GB-3123 and GB -7616X to Dr A. D. Hasler) and the Department of Zoology and Entomology, Montana State University.

REFERENCES

BATSCHELET, E. 1965. Statistical methods for the analysis of problems in animal orientation and certain biological rhythms. American Institute of Biological Sciences, Washington, D.C. 57 p.

COPE, O. B. 1957. Races of cutthroat trout in Yellowstone Lake. U.S. Fish Wildlife Serv. Spec. Sci. Rept. Fish. 208: 74–84.

GRIFFIN, D. R. 1952. Bird navigation. Biol. Rev. 27: 359–393.

HASLER, A. D., E. S. GARDELLA, R. M. HORRALL, AND H. F. HENDERSON. 1969. Open-water orientation of white bass, *Roccus chrysops*, as determined by ultrasonic tracking methods. J. Fish. Res. Bd. Canada 26: 2173–2192.

HASLER, A. D., R. M. HORRALL, W. J. WISBY, AND W. BRAEMER. 1958. Sun-orientation and homing in fishes. Limnol. Oceanogr. 3: 353–361.

HENDERSON, H. F., A. D. HASLER, AND G. C. CHIPMAN. 1966. An ultrasonic transmitter for use in studies of movements of fishes. Trans. Amer. Fish. Soc. 95: 350–356.

JAHN, L. A. 1966. Open-water movements of the cutthroat trout (*Salmo clarki*) in Yellowstone Lake after displacement from spawning streams. J. Fish. Res. Bd. Canada 23: 1475–1485. 1969. Movements and homing of cutthroat trout (*Salmo clarki*) from open-water areas of Yellowstone Lake. J. Fish. Res. Bd. Canada 26: 1243–1261.

MCCLEAVE, J. D. 1967. Homing and orientation of cutthroat trout (*Salmo clarki*) in Yellowstone Lake, with special reference to olfaction and vision. J. Fish. Res. Bd. Canada 24: 2011–2044.

MCCLEAVE, J. D., L. A. JAHN, AND C. J. D. BROWN. 1967. Miniature alligator clips as fish tags. Progr. Fish-Cult. 29: 60–61.

Behaviour of Lobsters Exposed to Bleached Kraft Mill Effluent

D. W. McLeese

Fisheries Research Board of Canada
Biological Station, St. Andrews, N.B.

McLeese, D. W. 1970. Behaviour of lobsters exposed to bleached kraft mill effluent. J. Fish. Res. Bd. Canada 27: 731–736.

In laboratory tests, lobsters (*Homarus americanus*) did not avoid bleached kraft mill effluent (BKME) at concentrations up to 20%. BKME or fresh water introduced 50 cm upstream from resting lobsters did not elicit behavioural responses. Lobsters may have detected at least two (acetic acid and NH_4OH) of six toxic or malodorous materials but did not avoid any of them. Based on these results, it seems unlikely that exposure to dilute solutions of BKME in nature would have immediate, direct effects on movement of lobsters.

Received November 17, 1969

INTRODUCTION

A BLEACHED KRAFT MILL near Pictou, Nova Scotia, which began production in September 1967, discharges bleached kraft mill effluent (BKME) at the rate of about 20 million gal per day (91,200 m^3) into a retention pond. Engineers of the Nova Scotia Water Resources Commission estimate that the effluent is retained in the pond for 40 days before it flows into the sea. Fishermen, disturbed by declining catches in the area, wondered if lobsters were being adversely affected by the BKME (Scarratt, MS, 1969).

Prior to the opening of the mill, Sprague and McLeese (1968) showed that survival of lobster larvae was not affected by a mixture of the three major components of BKME at concentrations below 10%, and also that adult lobsters survive at high concentrations of the BKME mixture (20–32%), but other aspects of the lobster's behaviour were not investigated. For lobsters in nature to encounter 32% BKME, they would be in areas of about 20‰ salinity. Newly hatched lobster larvae avoid water of 21.4‰ salinity and lower, and would probably avoid relatively high concentrations of BKME because of the associated lowered salinity (Scarratt, 1967).

This study was undertaken to determine if BKME solutions affect the behaviour of lobsters. For comparison, the reactions of lobsters to six toxic or malodorous materials were also observed.

MATERIALS AND METHODS

BKME was collected in June and July 1969 at the spillway to the sea from the retention pond at Boat Harbour near Pictou, N.S. The BKME was brought to St. Andrews, N.B., on the day of collection and stored at 5 C for 2 weeks or less until used in experiments.

Printed in Canada (J1648)

Intermoult lobsters of both sexes, weighing about 450 g each, were held in fiberglass tanks supplied with flowing sea water and were maintained and tested at ambient temperature (11–14 C). They were fed squid, fish muscle, and beef liver three times per week but food was withheld for 2 days prior to experiments.

In the test trough (Fig. 1) lobsters released in the central area encountered a seawater–test solution boundary regardless of direction of travel. The trough was made of untreated marine plywood. A suitable flow pattern was obtained with a continuous flow of sea water, about 2 liters/min, to the bottom of the central section. The water rose through the central area of the screen

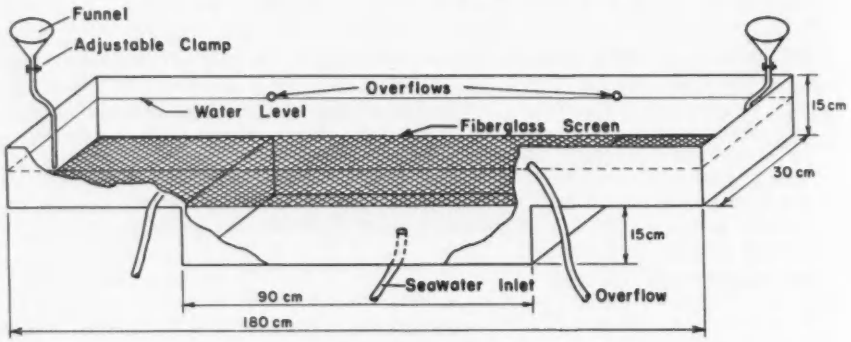


FIG. 1. Test trough.

walkway and flowed towards the ends to the outlets. The volume of each end zone was 13.5 liters. From 0.5 to 2.7 liters of test solution were added to each end zone over a period of 3–5 min. Tests with dye showed that vertical boundaries between sea water and test solutions became established at the overflow positions; that is, one-quarter of the length of the trough from each end. After about 17 min, the boundaries began to shift towards the ends but remained well defined for at least 28 min.

A few tests were run with a single test boundary established one-quarter of the trough length from one or other of the ends. Also there were a few tests with the boundary at the centre of the trough. For the latter, sea water flowed into one end of the trough, test solution into the other, both flowing out through central overflows.

After each test the trough was drained and flushed with sea water.

The apparatus was shielded with black plastic sheeting and lobsters were observed from a distance in a full-length mirror mounted at an angle over the trough.

When released in a tank such as the trough, lobsters usually explore it thoroughly for 20 min or more before settling at an end or corner. The animal was placed in the central area of the trough and confined between removable partitions, 30 cm apart, for about 10 min. During this time test solutions were added and test boundaries became established. The animal was then released by removing the partitions. In control tests with sea water, the animals remained active during the first 15 min after their release. When left longer, activity gradually subsided and the animals came to rest in an end zone, usually within 25–30 min. To ensure that the animals were active during the experiments, observations were made only during the first 5–10 or occasionally 15 min after release. Movement of the animal was recorded by hand on a kymograph.

Since each end zone occupied one-quarter of the area of the trough, active lobsters would normally be expected to spend 50% of the time in the end zones. However, because of time required to turn, the proportion of time would exceed 50%. In control tests with sea water, the lobsters spent 60–65% of the time in the end zones when tested within the first 15 min after their release (Table 1, Fig. 2A). Consequently in tests, less than 60% of the time in the end zones would in-

dicate avoidance. Similarly with test material in one end only, less than 30% would indicate avoidance.

In addition to considering the proportion of time spent in the various areas of the trough while the animals were active, their behaviour as they approached boundaries between sea water and test solutions was also observed. Reversal of direction by turning and walking in the opposite direction, or by rapid backward swimming, was considered to be an avoidance reaction.

Test concentrations of 10–20% BKME were obtained by adding 1.35–2.7 liters of the aqueous BKME solution (100% BKME) to each end of the trough. Other liquids (glacial acetic acid, NH_4OH, formalin, pyridine, and putrid cod extract) were tested at about 0.1% by adding 1 liter of sea water containing 13.5 ml of the test material to each test zone. Zinc chloride was tested at about 0.05% by adding 500 ml of 0.1 N solution.

Because of low density, the solutions of BKME, particularly 20%, tended to rise and spread over the surface. Since BKME solutions are brown, it was possible to determine if or when observations had to be terminated because of indistinct boundaries.

RESULTS AND DISCUSSION

Typical records for each test material (Fig. 2) illustrate the general activity of lobsters in the apparatus. The lobsters were considerably less active than young Atlantic salmon in an avoidance apparatus (Sprague, 1964) but were about as active as amphipods, *Gammarus pulex* (Costa, 1966).

In 13 tests with 10–20% BKME in both ends of the trough, lobsters spent an average of 77% (range 65–89%) of the time in the end zones (Table 1, Fig. 2H–J). In two tests with 10% BKME in half the trough, the mean time in the zone with BKME was 32% (20–54%). The mean time in the BKME zones for all 15 tests was 72%. In four other tests, two with BKME in both ends and two with BKME in half the trough, the animals walked into the BKME zones shortly after release but came to rest without further walking.

In 103 approaches to the seawater–BKME boundaries, the animals passed through the boundary 98 times, reversing direction in only 5 approaches. One of the reversals occurred in 19 initial approaches. After the reversals, the animals proceeded through the boundary when they next encountered it.

Considering the few reversals of direction when boundaries were approached and the high proportion of time spent in the end zones with BKME, there is little or no indication that the lobsters were avoiding the BKME solutions.

In the tests where lobsters spent 80% or more of time in the BKME zones, it was noted that the animals, on occasion, paused at the ends, which added to the time spent in the test zones. However, the possibility that the lobsters were attracted to BKME, or the associated lowered salinity, or both, was tested in an olfactometer (McLeese, 1969). Test solutions of 10 and 25 ml were introduced about 50 cm upstream from resting lobsters. Movement of mouthparts, sweeping motion of the first pair of walking legs, and walking upstream to the area where the samples were introduced indicate detection and attraction. These responses were observed in 95% or more of tests with 10 ml of dilute fresh cod extract (McLeese, 1969). However, no responses were observed in 27 tests with BKME or in 18 tests with fresh water, suggesting that these materials did not elicit behavioural responses.

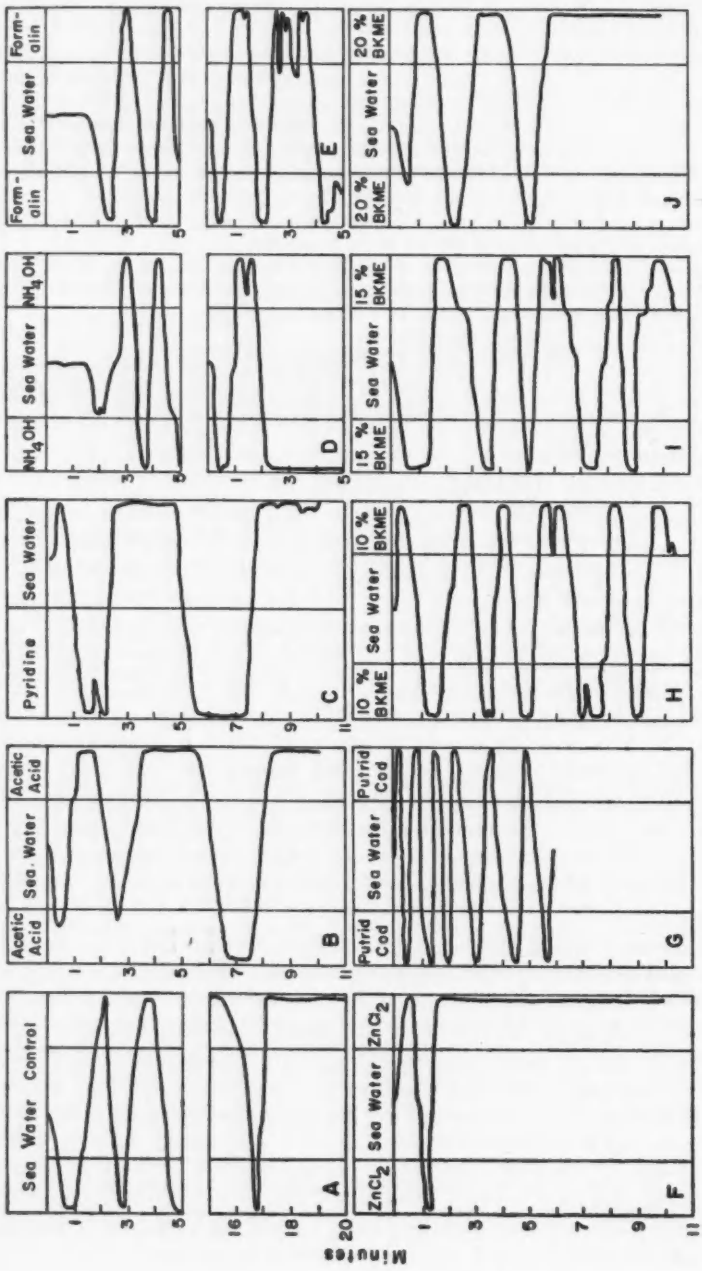


FIG. 2. Examples of lobster activity in the test trough with various materials present.

TABLE 1. Percentages of time lobsters spent in test zones and number of approaches and reversals of direction at seawater–test material boundaries. All tests were completed within 15 min after release of animals.

Material	No. trials	Total time (*min*)	Time in test zone		No. of boundary	
			Mean (%)	Range (%)	approaches	reversals
Sea water	3	17	62	(60–65)	14	0
Sea water[a]	3	15	97	(90–100)	2	0
BKME 10%	5	45	77	(65–85)	30	0
BKME 15%	5	50	76	(70–87)	36	3
BKME 20%	3	30	80	(76–89)	25	1
BKME 10%[b]	2	15	32	(20–54)	8	1
Acetic acid	4	35	65	(40–86)	21	3
NH_4OH	4	10	76	(46–94)	10	1
NH_4OH[b]	5	24	63	(0–88)	9	3
Formalin	6	28	68	(54–78)	23	0
$ZnCl_2$	8	42	91	(78–94)	19	1
Putrid cod	1	6	61	–	12	0
Pyridine[c]	2	15	57	(40–91)	3	0

[a]Sea water 15–35 min after release.
[b]Test material in one end (25% of area).
[c]Test material in half of trough.

Holland et al. (1960) found that small salmon (*Oncorhynchus kisutch*) in sea water did not avoid harmful concentrations of kraft waste effluent and that fish could not be driven from familiar stations in the experimental trough by concentrations of 3.5%. Sprague and Drury (1970) found that Atlantic salmon parr showed only moderate avoidance of BKME at concentrations up to 10%. Because of the weak avoidance reaction at concentrations up to 10% BKME, the authors concluded that salmon in nature would not necessarily avoid almost lethal levels (about 15%) of BKME.

With the various toxic or malodorous materials, the mean time lobsters spent in the two end zones was 65% for acetic acid, 76% for NH_4OH, 68% for formalin, 91% for $ZnCl_2$, and 61% for putrid cod extract. McLeese (1969) showed that lobsters in an olfactometer did not respond to putrid cod extract. In five tests with NH_4OH in one or other of the ends (25% of area), the mean time in the test zones was 63%. With pyridine in half the trough, the mean time in the test zone was 57% (Table 1, Fig. 2B–G).

There were no reversals of direction in a total of 38 approaches to the boundaries with formalin, putrid cod, and pyridine (Table 1). There were 3 reversals of direction in 21 approaches to the boundary with acetic acid, 4 reversals in 19 approaches with NH_4OH, and 1 in 19 approaches with $ZnCl_2$. Of these eight reversals, six occurred on the first encounter with the boundary. One reversal with acetic acid and one with NH_4OH were violent and both were accomplished by rapid backward swimming. These reversals show that at least some of the lobsters detected these materials; however, they invariably passed through the boundary on the next encounter.

Considering the few reversals that occurred with these materials and that mean time spent in the test zones was about equal to or greater than expected for all materials, there was no indication of avoidance.

Although amphipods (Costa, 1966), young Atlantic salmon (Sprague, 1964), and young rainbow trout (Sprague, 1968) avoid zinc, there is little or no indication that lobsters do so.

After penetration into formalin, pyridine, NH_4OH, and $ZnCl_2$ solutions, the lobsters became sluggish and in some instances immobile. For example, the animals became immobilized and were unable to leave the test zones in seven of nine tests with NH_4OH, seven of eight with $ZnCl_2$, and in one of two with pyridine. Costa (1966) observed similar immobilization with an amphipod when it crossed boundaries "without any outward hesitation" into chloroform or formalin and termed the apparent attraction a pseudopositive reaction.

Based on these laboratory experiments, it seems unlikely that exposure to dilute solutions of BKME in nature would have any immediate, direct effect on movement of lobsters.

ACKNOWLEDGMENTS

Thanks are extended to Messrs R. K. Robicheau and N. J. McFarlane for collecting the BKME and to the officers of the Department of Fisheries and Forestry, Pictou, N.S., for their assistance with the collections. Drs J. B. Sprague, P. E. K. Symons, and D. G. Wilder are thanked for their helpful criticisms of the text.

REFERENCES

Costa, H. H. 1966. Responses of *Gammarus pulex* (L.) to modified environment. I. Reactions to toxic solutions. Crustaceana 11: 245–256.

Holland, G. A., J. E. Lasater, E. D. Neumann, and W. E. Eldridge. 1960. Toxic effects of organic and inorganic pollutants on young salmon. Wash. Dep. Fish. Res. Bull. 5: 264 p.

McLeese, D. W. 1969. Detection of chemical odours by the lobster (*Homarus americanus*). Int. Counc. Explor. Sea, Gear Behaviour Comm. C.M. B-17: 1–7.

Scarratt, D. J. 1967. Avoidance of low salinity by newly hatched lobster larvae. J. Fish. Res. Bd. Canada 24: 1403–1406.

MS, 1969. Bleached kraft mill effluent near Pictou, N.S. and its effect on the marine flora and fauna with a note on the Pictou Co. lobster landings. Fish. Res. Board Can. MS Rep. 1037: 33 p.

Sprague, J. B. 1964. Avpidance of copper–zinc solutions by young salmon in the laboratory. J. Water Pollut. Contr. Fed. 36: 990–1004.

1968. Avoidance reactions of rainbow trout to zinc sulphate solutions. Water Res. 2: 367–372.

Sprague, J. B., and D. E. Drury 1970. Avoidance reactions of salmonid fish to representative pollutants. J. Water Pollut. Contr. Fed. (In press.)

Sprague, J. B., and D. W. McLeese. 1968. Toxicity of kraft pulp mill effluent for larval and adult lobsters, and juvenile salmon. Water Res. 2: 753–760.

A Comparison between Two Sonic Measuring Systems for Demersal Fish[1]

R. G. DOWD

Fisheries Research Board of Canada
Marine Ecology Laboratory
Bedford Institute, Dartmouth, N.S.

AND

E. BAKKEN AND O. NAKKEN

Institute of Marine Research
Bergen, Norway

DOWD, R. G., E. BAKKEN, AND O. NAKKEN. 1970. A comparison between two sonic measuring systems for demersal fish. J. Fish. Res. Bd. Canada 27: 737–742.

Two sonic methods for estimation of abundance of fish stocks, the echo integrator and the digital counter methods, were compared on single and schooling fish in the Lofoten area of Norway during March 1969. Good correlation was obtained between the two systems for both situations, but the slopes of the regressions of integrated values on the digital counter differed significantly between low and high density fish concentrations. This suggests that the two systems treated the echo information differently, but nevertheless maintained a linear relation between themselves over a wide range of counts.

Received January 23, 1970

INTRODUCTION

TWO MAIN PROBLEMS ARISE when acoustic instruments are used to estimate fish abundance. The first is to obtain reliable counts of the number of fish per unit volume detected by the echo sounder. Different systems have been developed for this purpose (Dragesund and Olsen, 1965; Dowd, 1967; Midttun and Nakken, 1968). The second is to determine the effective volume of water sampled uniquely by each sonic pulse (Cushing, 1968). For the latter purpose, it is necessary to know the target strength of the fish. Recently, Craig and Forbes (1969) have described a sonar for fish counting and derived an excellent method for determining the number of fish counted per unit volume.

The experiment reported here is confined to problems of the first kind and was carried out to compare two kinds of signal processing systems, the Digital Echo Counter (Dowd, 1967) and an Analog Echo Integrator (Dragesund and Olsen, 1965). The main questions to be answered were: is there a linear

[1]Bedford Institute Contribution No. 190.

Printed in Canada (J1699)

relation between systems when they are sampling signals from the same "isonified" volume, and can these signals be correlated with a count of echos obtained from echo-sounder paper recordings? The two systems were compared at different pulse lengths with reflections from various densities of spawning cod (*Gadus morhua* L.). The work was done on board the Norwegian Research Vessel *G. O. Sars*, in Lofoten, Norway, in early March 1969.

EQUIPMENT AND METHOD

The equipment was set up as in Fig. 1. A Simrad Model EK38 scientific echo sounder was used with a transmitted power of 1000 w and a measured source level of +118 db/μ bar at 1 m, emitted from the 15 × 30-cm transducer. The receiver has a time varied gain (T.V.G.) function and was set to compensate two-way transmission loss down to 230 m. The output of the receiver was injected into the three recording systems: the echo-sounder recorder, the echo integrator, the digital echo counter. The threshold for all three was set at −24 dbv to permit a direct comparison between the systems.

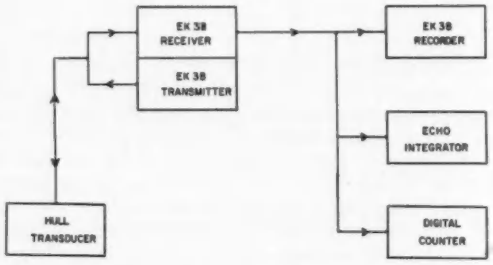


FIG. 1. A simplified block diagram of the system used on research vessel *G. O. Sars*. Arrows show direction of signal in the system.

The echo integrator (Dragesund and Olsen, 1965) accumulates the voltage from the received echos and resets automatically after each nautical mile or after full-scale deflection is reached. Also, it can be reset at any time by the operator. The sampling depth range is controlled from the transmitted pulse, gating for the desired layer to be sampled. If individual fish are recorded, the integrator reading is proportional to the number of recorded fish.

The echo-counting system (Dowd, 1967) records the number of echos from each transmission in digital form. The system uses a delayed pulse technique to sample a fixed distance from bottom irrespective of water depth. The resolution obtained depends on the pulse length, and separate counts can be obtained for individual fish if they are separated by more than 25 cm. In this experiment the echo counter was changed to sample the same layer as the integrator and converted from delayed pulse to transmit gate. The pulse lengths used were 0.3, 0.6, and 1.0 msec, nominal. Since the integrator was not changed to compensate for change in pulse lengths and the pulse-length gates in the counting system were changed to 0.6, 0.9, and 1.3 msec respectively, an increase in slope with an increase in pulse length was expected.

In Lofoten, the spawning cod are concentrated in a layer between 75 and 125 m below the surface and where the depth of water is approximately 150 m. During the night the cod occurred as well-separated fish, but aggregated in shoals during the day. In a sample obtained by purse seine the mean length was found to be 89 cm. The length of cod was generally between 65 and 105 cm. The sampling of echo information was carried out mostly during the night hours, and areas of different concentrations were selected. The ship speed was maintained at 5 knots. The counter and integrator were adjusted to record signals from the same depth range, 100–125 m. The readouts were taken at 10-min intervals. Echo-sounder paper counts for the depth range were taken whenever separation of echos permitted.

RESULTS

Figures 2A, B, and C show plots of integrator voltage versus echo count with fitted regression lines. The correlation coefficients are 0.84 (24 df), 0.94 (30 df), and 0.96 (30 df), for the pulse lengths of 0.3, 0.6, and 1.0 msec respectively. Since the integrator voltage is directly proportional to the pulse length and the counting system was adjusted to compensate for this change, an increase in slope with an increase in pulse length was expected and obtained. The slope for the three pulse lengths differed significantly as follows:

Pulse length (*msec*)	Slope	Difference between pulse lengths	t	df
0.3	.0044	0.3–0.6	3.7	52
0.6	.0070	0.3–1.0	5.7	53
1.0	.0085	0.6–1.0	2.7	53

Figure 2D shows the relation between the integrator and counter for high density daytime recording. The correlation coefficient between the two for the 0.3-msec pulse length was 0.99 (14 df). However, there was significant difference between the slopes for the day and night recordings (i.e., high and low densities) for 0.3-msec pulse lengths (t = 4.1, 37 df).

Figures 3A, B, and C show the relation between the actual paper counts and converted digital counts. Because individual counts cannot be resolved on paper for high fish densities, the comparison was possible only for night recordings.

To convert the digital instrument counts, C, into the estimated numbers of targets (fish), N, we proceeded as follows. If b_w is the beam width at the sampling depth and t_r is the transmission rate, if V is the ship's speed and T is the time of run, then the overlap factor between successive transmission is $(\frac{b_w t_r}{VT})$. The estimated number of targets seen by the counting system during the sample period is then

$$N = C\left(\frac{VT}{b_w t_r}\right).$$

With this formula, a conversion to the estimated numbers of fish was obtained and plotted against the paper counts (Fig. 3). Good correlation was obtained between the two methods with correlation coefficients of 0.96 (23 df), 0.98 (29 df), and 0.91 (30 df) for the pulse length of 0.3, 0.6, and 1.0 msec respectively.

DISCUSSION

The integrator readings and the echo counts for data of Fig. 2A–D could have been converted to the actual numbers of fish since the target strength of cod is known. This is unnecessary when comparing the two measuring

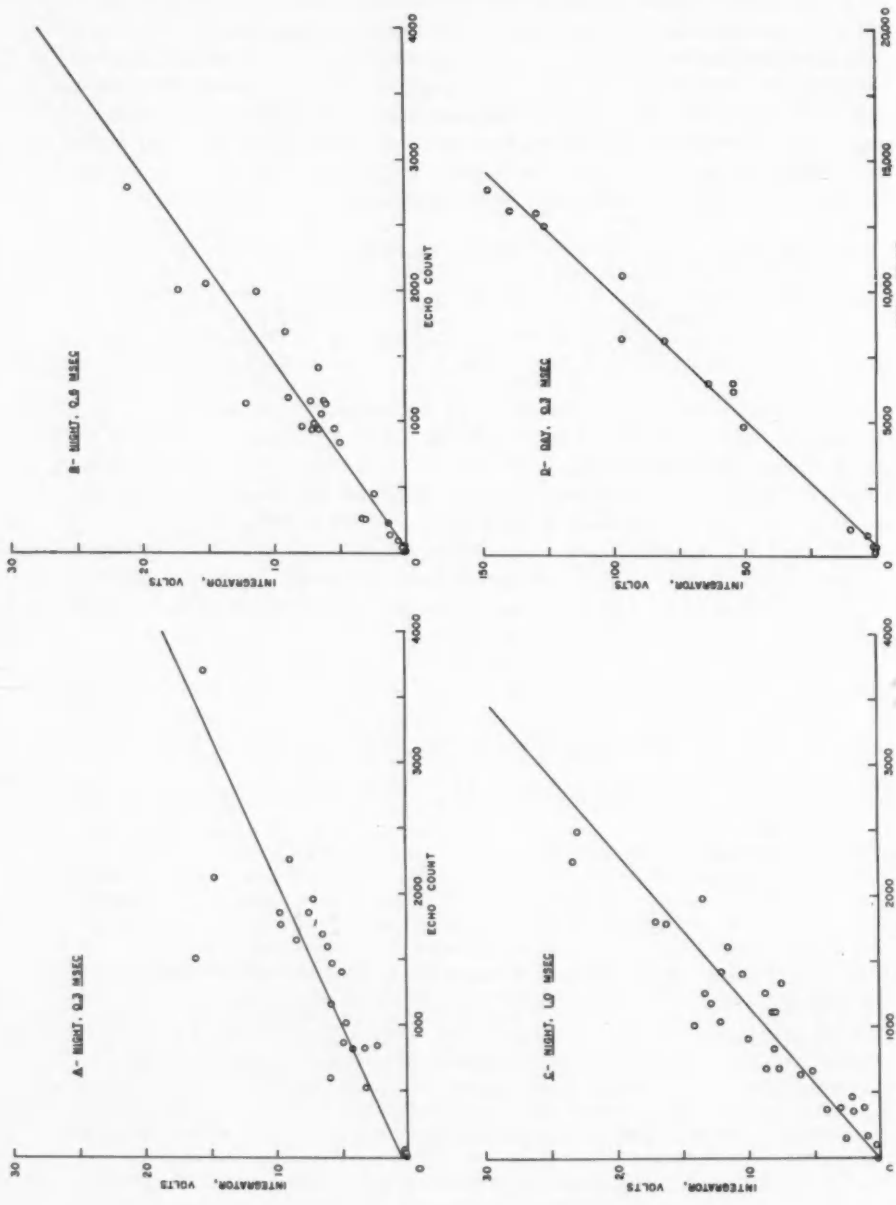


FIG. 2. Relation between integrator voltage and digital echo counts for: (A) 0.3 msec, (B) 0.6 msec, (C) 1.0 msec, night recording, and (D) 0.3 msec, day recording. The scale for D has been extended but maintains the same X–Y ratio as A, B, and C.

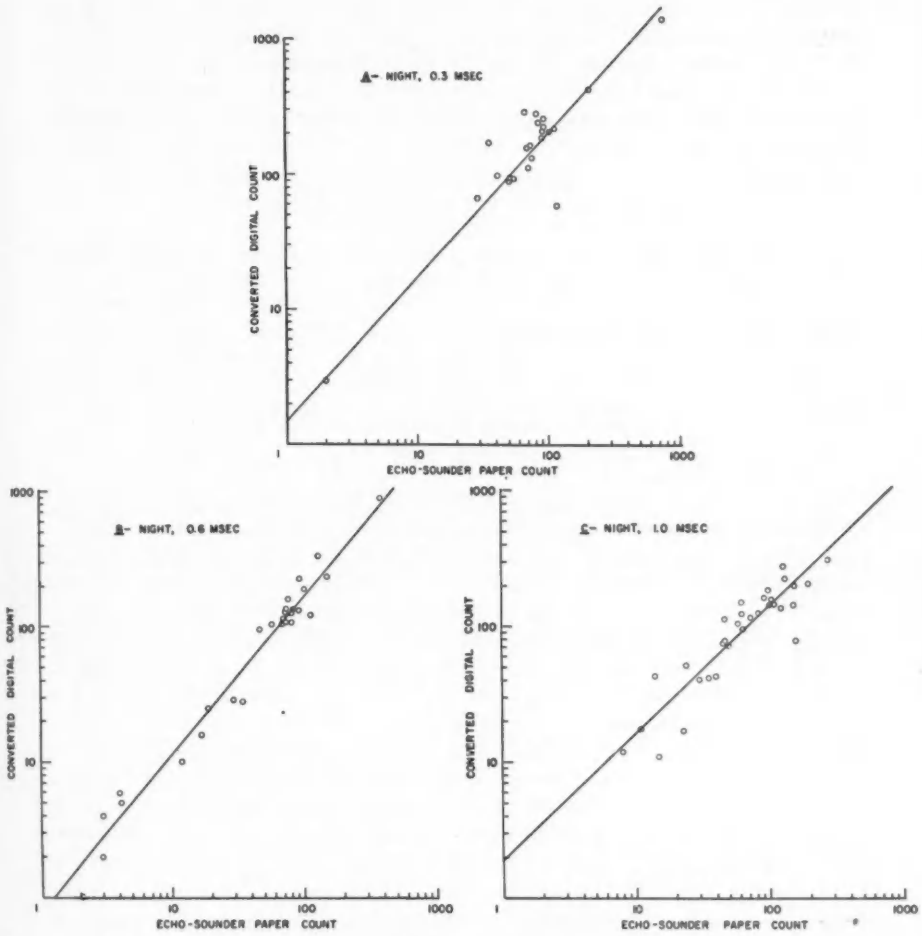


FIG. 3. Relation between converted digital echo count and echo-sounder recorder paper count for: (A) 0.3 msec, (B) 0.6 msec, and (C) 1.0 msec.

systems. At low fish densities, good agreement was found in all instances among the three systems. However, the relation between the number of counts and the integrator values was found to be significantly different between day and night. This implies that one or both of the recording systems are not linear with the actual numbers of fish. At night, when fish are recorded as singles, both units record values proportional to the actual numbers. But by day, when the fish are aggregated, the digital recorder accumulates lower numbers in relation to the integrator. However, the relation between the two systems

FIG. 2. Relation between integrator voltage and digital echo counts for: (A) 0.3 msec, (B) 0.6 msec, (C) 1.0 msec, night recording, and (D) 0.3 msec, day recording. The scale for D has been extended but maintains the same X-Y ratio as A, B, and C.

is linear over a wide range of total counts, implying that the density of aggregation is reasonably constant and independent of school size. It also implies that neither system has a linear response to changes in the total actual numbers of fish during schooling. Further experiments should be conducted on the aggregation behaviour of fish for stock estimation, including integration, counting, and fishing, to attempt to obtain a better knowledge of the relation between sonic measurements and the actual numbers of fish is schools.

ACKNOWLEDGMENTS

We thank Mr L. Midttun of the Institute of Marine Research, and the officers and crew of the Norwegian Research Vessel *G. O. Sars* for their assistance in making the experiment possible. Also we thank Dr L. M. Dickie, Dr R. W. Trites, and Mr T. Platt whose criticism and assistance, during writing, were invaluable.

REFERENCES

CUSHING, D. H. 1968. The abundance of hake off South Africa. Fish. Invest. Min. Agr. Fish. Food (Gt. Brit.) Ser. II. Salmon Freshwater Fish. 25(10): 1–20.

CRAIG, R. E., AND S. FORBES. 1969. A sonar for fish counting. Fiskeri Dir. Skr. Ser. Havunders. 15: 210–219.

DOWD, R. G. 1967. An echo counting system for demersal fishes. FAO Conference on fish behaviour in relation to fishing techniques and tactics. FB/67/E/7: 1–6.

DRAGESUND, O., AND S. OLSEN. 1965. On the possibility of estimating year-class strength by measuring echo-abundance of 0-group fish. Fiskeri Dir. Skr. Ser. Havunders. 13(8): 47–75.

MIDTTUN, L., AND O. NAKKEN. 1968. Counting of fish with an echo-integrator. Int. Counc. Explor. Sea B17: 1–7.

Effects of Light on Spat Settlement of the American Oyster (*Crassostrea virginica*)

ROSSLYN SHAW,[1] DAVID C. ARNOLD, AND WILSON B. STALLWORTHY

Department of Biology
Mount Allison University, Sackville, N.B.

SHAW, R., D. C. ARNOLD, AND W. B. STALLWORTHY. 1970. Effects of light on spat settlement of the American oyster (*Crassostrea virginica*). J. Fish. Res. Bd. Canada 27: 743–748.

Comparing numbers of spat obtained in opaque tanks with those from transparent tanks where time of illumination was varied, indicated that setting of mature larvae of *Crassostrea virginica* Gmelin is encouraged by darkness and partially inhibited by light.

Received January 23, 1970

INTRODUCTION

IN SPITE OF the continuing interest in the American oyster, *Crassostrea virginica* Gmelin, very little work has been done on the sensitivity of its mature larval form to light.

Thorson (1964) summarized the conclusions drawn from the few reports in the literature with regard to the effect of light in the dispersal and settlement of the pelagic larvae of bottom-dwelling invertebrates. He states that Nelson (1926) found that the pallial eyes of the mature larvae of *C. virginica* are of value in causing them to set in shaded areas; Cole and Knight-Jones (1949) showed a marked preference on the part of the larvae of *Ostrea edulis* L. for the under surfaces of dark plates. During daylight, these larvae tended to select a shady situation for attachment. The larvae of *C. virginica* are photopositive in their early pelagic life (Nelson, 1916; Carriker, 1951; Bousfield, 1955), but this response soon changes to an indifference or a negative reaction in the latter part of their pelagic existence (Nelson, 1926; Bousfield, 1955). This interpretation is contrary to the conjecture of Prytherch (1934). It is also contrary to the conclusion that light stimulates setting, drawn by Medcof (1955) for larvae in the Bras d'Or Lake, Nova Scotia. According to Bayne (1964), a threshold of stimulation by light exists for both the swimming and crawling stages of the larval form of the blue mussel, *Mytilus edulis* L., and there also seems to be some evidence that this particular threshold changes with age. Since previous work on the American oyster has been carried out under a diversity of physical and chemical conditions, it is not possible to establish a standard as to what actually stimulates an oyster larva to settle.

The purpose of this investigation, which was carried out at the Fisheries Research Board of Canada hatchery, Ellerslie, Prince Edward Island, was to

[1]Present address: Gratty Marine Laboratory, St. Andrews, Scotland.

Printed in Canada (J1700)

document the effect of light as a factor in the settlement of the mature larvae of *C. virginica*, while recognizing the possibility that there might be a photoperiod effect in the settlement of oyster larvae. The problem was examined by observation of settlement in transparent and opaque tanks and also by varying illumination blackout times in transparent tanks.

MATERIALS AND METHODS

The work was undertaken in two sequences of experiments, with some differences in technique introduced into the later experiments as a result of experience. In earlier studies (summers of 1967 and 1968) investigations were restricted to comparison of opaque and transparent tanks of different form kept under normal lighting. However, in 1969 special attention was paid to the duration of illumination and procedure was modified accordingly. In all experiments temperatures were 21–23 C, salinity 26–27‰, water was air-stirred and known to be saturated with oxygen at all levels.

Opaque vs. Transparent Tanks under Normal Lighting

(*a*) The opaque tank was a small conical fiberglass tank of about 40 cm depth, and approximately 102,000 larvae were introduced at the start of the experiment. Collectors were scallop shells on nylon strings at 7.6-cm intervals and were presoaked 24 hr in running sea water before being placed for 24 hr in the experimental tank. The resulting spat were counted after placing a fresh string of soaked shells in the tank. This procedure was repeated on 3 successive days using the same water and larvae but different collectors.

(*b*) The transparent tanks were two plexiglass columns 5.1 cm square in section and 1.2 m in length. They were used in duplicate in one experiment and combined to make a column 2.4 m high in a second experiment. For observation at 1.2 m depth, 4000 mature larvae were introduced, setting was observed over 24 hr, and the same procedure repeated twice more. For the 2.4-m trial, 8000 mature larvae were added and setting was observed over 24 hr. This was repeated once. Collectors were pieces of scallop shell 2.5 cm square placed at 8.9-cm intervals on nylon cord.

Controlled Duration of Illumination

Setting activity was assessed every day for 3 or 4 days by counting the spat that appeared on standard collectors placed in experimental tanks, each containing a sample population of mature swimming larvae and subjected to controlled lighting conditions. Tanks were 43.8 × 22.5 × 28.1 cm and contained about 7.5 gal. Thermostats (Metaframe, Model 22) and air stones operated in each tank. Sea water in the tanks had been filtered through a 50-μ filter and passed through an ultraviolet sterilizer. Actual water conditions (temperature, salinity, and *p*H) for each of the two trials are shown in Table 2.

In the blacked-out laboratory, lighting was by two 4-ft cool daylight fluorescent bulbs close enough to the tanks to give an intensity of 450 ft-c, and also by a string of five 7.5-w incandescent bulbs giving 7.5 ft-c at the tanks. These were arranged on time switches so that a period of 2 hr at 7.5 ft-c preceded and also followed a period of full illumination from both sources at once. This sequence was an attempt to simulate natural twilight conditions. The actual periods of blackout used are given in Table 2. The tank with total blackout was wrapped in one layer of black plastic and one layer of aluminium foil throughout the trials, and an additional tank during each trial was left exposed to the natural light of the hatchery (north daylight).

Approximately 13,000 mature larvae were added to each tank at the beginning of each trial. Observations using a plankton microscope showed that all of the larvae were over 300 μ in diameter, each contained a very large eye, and many had their feet in a protruded state. Swimming activity of the larvae appeared to be normal. Each day, the larvae in each tank were fed 100 ml of *Dicrateria* algal culture. Each tank was also inoculated with an 0.5-ml aliquot of an antibiotic, bistrept, to lessen bacterial contamination.

At the termination of each trial, each tank was drained through a 243-μ screen to retain all of the larvae remaining in the water. These larvae were then placed in a bucket in 2 liters of water, a few drops of formalin were added and the bucket swirled so that all larvae clumped together on the bottom in a mass in the middle. All of the larvae in the clump were placed in a plankton counting chamber. The number of previously dead larvae were recognizable and could be counted under a plankton microscope.

Collectors consisted of squares of translucent fiberglass (7.6 × 7.6 × 0.15 cm) drilled at the center and suspended in strings of four in the water. Three such strings sampled each tank. All collectors were soaked in running (hatchery) sea water for 6 days before use. At the end of each 24-hr period during both trials all collectors were exchanged for freshly soaked ones, and the spat found on both upper and lower surfaces were recorded.

RESULTS

Preliminary experiments in 1967 showed clearly that light exerted an influence on setting. Comparison of spatfall in opaque and translucent tanks indicated that either increase in depth or decrease in light intensity resulted in an increased set. Further studies employing 2.4-m plastic columns revealed no influence of increasing depth alone as shown in Table 1.

TABLE 1. Spat found on shell collectors placed in a small conical tank and in a tall plexiglass column.

Small conical tank 1967							Tall plexiglass column 1968				
Level (*cm*)	June 18	19	20	22	23	Total	Level (*cm*)	June 4	5	6	Total
0	174	932	14	0	5	1125	0	52	60	52	164
7.6	689	777	123	1	28	1618	10.2	35	10	24	69
15.2	744	980	130	0	31	1885	20.3	22	28	150	200
22.9	414	657	201	5	36	1313	30.5	7	5	12	24
30.5	741	1681	173	5	80	2681	40.6	2	6	6	14
38.1	1037	2489	290	2	65	3883	50.8	2	3	72	77
							61.0	12	13	10	35
							71.1	10	0	10	20
							81.3	8	12	63	83
							91.4	11	4	52	67
							101.6	11	9	70	90
							111.8	6	10	29	45
							122.0	16	68	182	266
							On bottom	12	142	321	475

Actual spat counts obtained when illumination was controlled in transparent tanks are shown in Table 2 and are plotted as percent of live larvae setting in Fig. 1. Clearly, the tanks kept in total darkness had the most spat. Even though the two experiments are plotted on different scales the graphs strongly suggest a direct proportionality between duration of light and numbers of spat. Moreover, a steady increase in numbers of spat found as the days go

TABLE 2. Numbers of spat found on experimental collectors for various periods of blackout in 1969. Trial 1, larvae from Freeland commercial hatchery; trial 2, larvae from Ellerslie hatchery.

Hr of blackout/ 24-hr period	Trial 1[a]					Trial 2[b]			
	June 18	19	20	21	Totals	July 18	19	20	Totals
24	88	254	271	220	833	33	45	65	143
12	23	34	178	194	429				
8[c]	39	96	124	83	342				
7[c]						12	18	21	51
4						8	15	27	50
0						0	12	26	38

[a]Water temperature 25 C; salinity 28.4‰; pH 8.06.
[b]Water temperature 24 C; salinity 26.95‰; pH 8.10.
[c]Tanks were illuminated with artificial light, except for those with 8 and 7 hr blackout, which were in natural daylight in the hatchery.

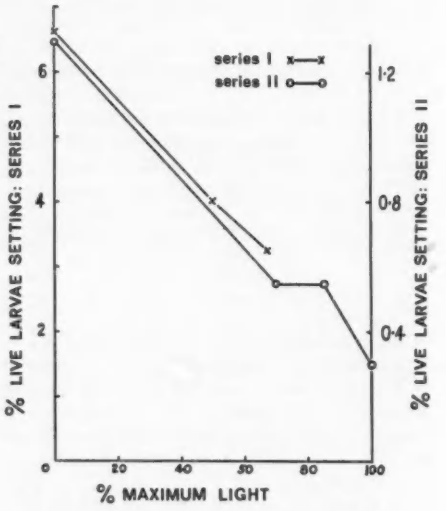


FIG. 1. Effects of light on numbers of oyster larvae setting. Total spat over 3–4 days expressed as percent of living larvae present, plotted against the proportion (percent) of each 24-hr period during which tanks were illuminated.

by is characteristic of both trials (Table 2). Average total mortality during trials was 2190 ± 32 out of the 13,000 larvae introduced, or 16.8%.

DISCUSSION

The data of all 3 years are consistent and leave no doubt that light inhibits setting behaviour in *C. virginica*, although the inhibition is not complete even in full illumination. For every additional hour of light per day, the set is decreased by approximately 1/30 of the set observed in complete darkness. Nevertheless, even in complete darkness the number of larvae setting in 3–4

days was never more than 7% of all the living larvae present, and in continuous light the total number setting remained less than 1% of all living larvae present.

These results directly oppose the finding of Medcof (1955) that light favours setting. This would seem to indicate that the effect of light in producing Medcof's results was indirect. Perhaps, as he suggests, it stimulates swimming or feeding, or both. This does, in any case, emphasize the complexity of the setting reaction and points also to conditions other than light that may have differed between the Bras d'Or Lake and the hatchery or laboratory. One of these might have been oxygen concentration, which was not checked by Medcof, but in our conical tanks was saturated at all levels. Also, the laboratory and hatchery tanks are all air-stirred, which provides a different stimulus and certainly alters the pattern of water currents. Distribution of food organisms might also have some influence.

Some observations in the literature are in agreement with our results, however. The selection by *C. virginica* of shaded areas (Nelson, 1926), the preference of *Ostrea edulis* for under surfaces of dark plates and shaded locations (Cole and Knight-Jones, 1949), and the negative photoreaction reported by Nelson (1926) and Bousfield (1955), the tendency of mature larvae to seek the deeper layers of water (Galtsoff, 1964, p. 370) are all, at least in the same sense, with regard to lighting conditions. It now seems probable, in view of the present work with controlled lighting, that light also has been a factor in the above reported observations.

Demonstration of a light (or dark) effect gives no indication of the mechanism of its action. Two contrasting mechanisms suggest themselves with the possibility that intermediates may exist. In the first, light would act as a stimulus to swimming and feeding, whereas diminished light intensity would be correlated with reduced activity, leading to crawling behaviour and the accumulation of larvae in shaded places. This behavioural pattern would then be *followed* by cytological and physiological changes involved in settlement and metamorphosis. In such an instance, increased duration of high illumination would diminish the set obtained but stimulate the remaining larvae to active feeding and growth.

Alternatively, physiological factors necessary for settlement might arise while the larvae are planktonic and prior to any form of setting behaviour. At the moment such physiological factors are impossible to characterize, but might involve either or both a threshold sensitivity of response of the tissues and a critical accumulation of specific chemical entities. In this instance it would be expected that the level and duration of illumination would act as a release for setting behaviour in an exactly similar manner, but that if the setting did not occur the "ready" larvae would then die, being no longer adequately adapted to planktonic life. In fact, the later experiments revealed a high correlation ($r = 0.93$, Pearson) between larval mortality and duration of high illumination, suggesting that the second hypothesis (physiological changes precede settlement behaviour) is correct.

CONCLUSIONS

Setting activity in mature larvae of *Crassostrea virginica* Gmelin is encouraged by dark and partially inhibited by light. The numbers of larvae setting in 24 hr appear to be proportional to the numbers of hours of dark provided, and the number of larvae dying is strongly correlated with the amount of illumination.

ACKNOWLEDGMENTS

The authors acknowledge and express thanks for support from various agencies and individuals. The Fisheries Research Board of Canada, Ellerslie, P.E.I., provided space, equipment, and oyster larvae. The commercial oyster hatchery at Freeland also supplied living oyster larvae. Financial support from the National Research Council, the Atlantic Provinces Inter-university Committee on The Sciences, and Mount Allison University, are all gratefully acknowledged. Finally, Professor D. S. Fensom has provided the necessary help and administrative support to maintain the continuity of this work.

REFERENCES

BAYNE, B. L. 1964. The responses of the larvae of *Mytilus edulis* (L) to light and to gravity. Oikos 15: 162–174.

BOUSFIELD, E. L. 1955. Ecological control of the occurrence of barnacles in the Miramichi Estuary. Nat. Mus. Can. Bull. 137: 69 p.

CARRIKER, M. R. 1951. Ecological observations on the distribution of oyster larvae in New Jersey estuaries. Ecol. Monogr. 21: 19–38.

COLE, H. A., AND E. W. KNIGHT-JONES. 1949. The setting behaviour of the larvae of the European flat oyster, *Ostrea edulis* L. and its influence on methods of cultivation and spat collection. Fish. Invest. Min. Agr. Fish. Food (Gt. Brit.) Ser. II. Salmon Freshwater Fish. 17: 39 p.

GALTSOFF, P. S. 1964. The American oyster, *Crassostrea virginica* Gmelin. Fish. Bull. 64: 480 p.

MEDCOF, J. C. 1955. Day and night characteristics of spatfall and of behaviour of oyster larvae. J. Fish. Res. Bd. Canada 12: 270–286.

NELSON, J. 1916. Report of the Department of Biology of the New Jersey Agricultural College Experimental Station, for the year ending Oct. 31, 1915. p. 237–260.

NELSON, T. C. 1926. Report of the Department of Biology of the New Jersey Agricultural Station for the year ending June 30, 1925. p. 281–288.

PRYTHERCH, H. F. 1934. The role of copper in the setting, metamorphosis and distribution of the American oyster, *Ostrea virginica*. Ecol. Monogr. 4: 47–107.

THORSON, G. 1964. Light as an ecological factor in the dispersal and settlement of larvae of marine bottom invertebrates. Orphelia 1: 167–208.

Action Spectrum of the Photoperiod Mechanism Controlling Sexual Maturation in the Threespine Stickleback, *Gasterosteus aculeatus*

JOHN E. MCINERNEY AND DAVID O. EVANS

Department of Biology
University of Victoria, Victoria, B.C.

MCINERNEY, J. E., AND D. O. EVANS. 1970. Action spectrum of the photoperiod mechanism controlling sexual maturation in the threespine stickleback, *Gasterosteus aculeatus*. J. Fish. Res. Bd. Canada 27: 749–763.

Experiments in which groups of 80 threespine stickleback (*Gasterosteus aculeatus*) were exposed to four discrete segments of the visible spectrum (388–466, 455–518, 513–583, and 585–653 mμ) and compared with two control groups (experimental and wild) showed no major differences in seasonal rates of gonad maturation. This result contrasts with the narrower action spectra of the photoperiod mechanisms in some birds (600–750 mμ) and many insects (400–500 mμ). Light energy levels of $< 0.37 \times 10^3$ erqs/cm²-sec (equivalent to approximately 5 lux at 420 mμ) induced normal seasonal changes indicating a level of sensitivity for the photoperiod mechanism in this species comparable to that of certain birds and insects (approximately 1 lux). Histological comparisons of ovary development with the brook stickleback (*Culaea inconstans*) showed closely similar development patterns except for the final stage of yolk deposition. A further stage of yolk differentiation described for the threespine stickleback probably accounts for the larger egg size in this species.

Received November 28, 1969

INTRODUCTION

CHANGING PHOTOPERIODS acting independently or in concert with other environmental factors have been shown to control seasonal changes in the physiology and behaviour of animals in temperate latitudes (Farner, 1958; Harrington, 1959; Wolfson, 1964). Less attention has been given to the qualities of light as the effective stimulus. For example light intensity and spectral distribution might be expected to depend on specific photoreceptor characteristics.

The purpose of this study was to test the effects of selected wavelength segments of the "visible" spectrum on photoperiod-induced sexual maturation of the threespine stickleback (*Gasterosteus aculeatus* L.). Baggerman (1957) has shown that gonad maturation in this species depends on the increasing day lengths of late winter and spring; also, that in the absence of long photoperiods, high temperature (20 C) is not effective in inducing sexual maturation but that combined with long photoperiods, high temperatures accelerate the maturation process.

Although the spectral absorption characteristics of fish scotopic pigments have been documented phylogenetically (Crescitelli, 1958), little information is available for photopic receptors. Considering, however, the importance and diversity of male colouration in stickleback reproductive behaviour

Printed in Canada (J1656)

(Tinbergen, 1951; McInerney, 1969), it is reasonable to assume that colour vision is well developed in the family Gasterosteidae. Indeed the photoperiod response may be mediated by encephalic receptors rather than those of the retina (Farner, 1961).

Information on the action spectrum of the photoperiod mechanism in vertebrates is apparently limited to birds (Farner, 1961) and indicates that wavelengths in the red and orange region are effective in inducing gonadal maturation whereas shorter wavelengths (blue and green) are ineffective.

MATERIALS AND METHODS

Six groups of sticklebacks were studied. A wild (control) group was collected periodically from the Gorge (Tillicum bridge), a body of water connecting Victoria Harbour with Portage Inlet. It was not established whether this was a resident or seasonally changing population. The other five groups (approximately 80 in each) were collected in the same area on January 2, 1969, and housed in experimental light boxes (January 3–June 23, 1969).

The experimental groups were maintained in 20-gal aquaria in continuously filtered water (glass wool and charcoal) that was changed at approximately 4-week intervals. Food, consisting mainly of frozen brine shrimp supplemented occasionally with Daphnia and a flake type tropical fish food (Vitawil), was supplied twice daily. Less than 2% mortality was recorded.

The environmental regimes for each group are shown in Fig. 1, except for the wild (control) group, which was exposed to natural conditions (temperatures from 3 to 20 C and salinities from 18 to 24‰). The five experimental groups were maintained under as nearly identical conditions as possible, except for different light wavelengths.

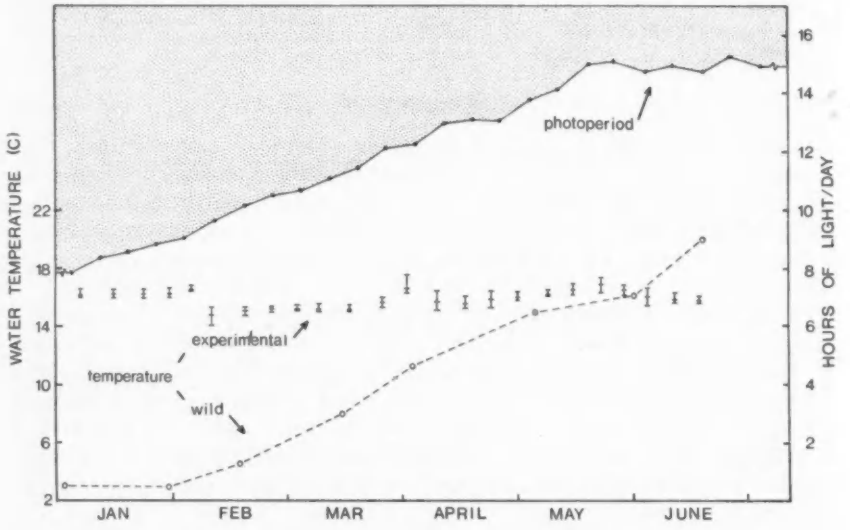


FIG. 1. Seasonal changes in photoperiod and water temperatures.

In each experimental group temperatures were maintained at 16 ± 1.5 C by means of submerged thermostatically controlled 100-w heaters. Salinities were adjusted with sea water to approximately 1‰ to maintain an adequate ion supply in the otherwise soft Victoria freshwater

supply. Light intensities were maintained at 0.37×10^3 erqs/cm^2-sec $\pm$ 0.08 by means of neutral density filters and a fluorescent dimming system (luxtrol L.F.D. 10, American Superior Electric Co.). Light intensity was measured with a factory-calibrated Yellow Spring Instrument, Kettering radiometer (thermistor-bolometer), equipped with a polished aluminum collecting cone. Measurements were made 1.5 cm below the light filter sandwiches located over each tank.

The five different wavelength regimes used are shown in Fig. 2. General Electric daylight fluorescent tubes were selected as the light source because of their relatively balanced output across the visible spectrum (General Electric Bulletin T.P. 111 on fluorescent lamps). A plywood panel containing five filter sandwiches (three directly above each aquarium and two in front) separated six fluorescent lamps (three over each group of filters) from the otherwise light-proof

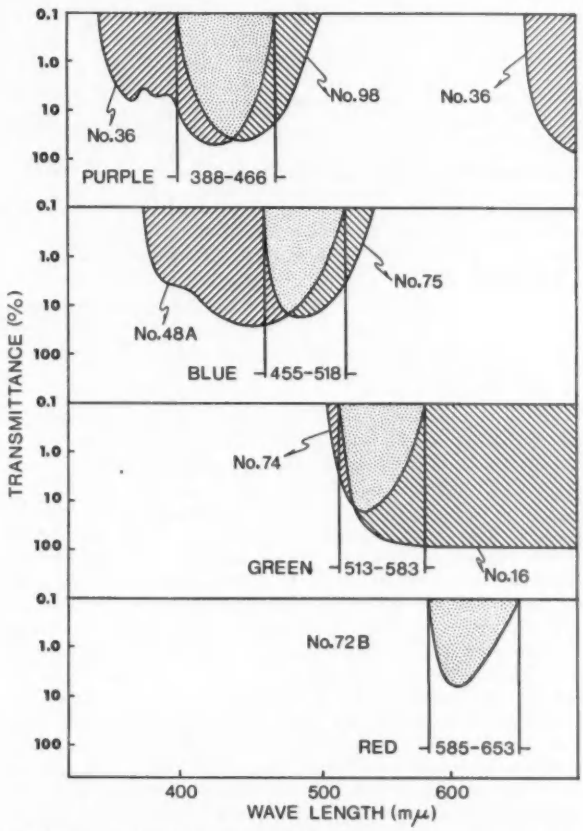


FIG. 2. Experimental wavelength bands showing the Kodak Wratten filter combinations employed. Hatched lines show the "window(s)" in the individual filters; the stippled areas show the "window" in the combined filter sandwich. (Data from Kodak publication B-3, Kodak Wratten Filters, revised 1968.)

chambers housing the aquaria (Fig. 3). The filter sandwiches each consisted of two outer sheets of 5/64-inch glass enclosing one or two Kodak wratten gelatine colour filters and, except for the red box, a Kodak gelatine neutral density filter. The colour filter combinations for each group

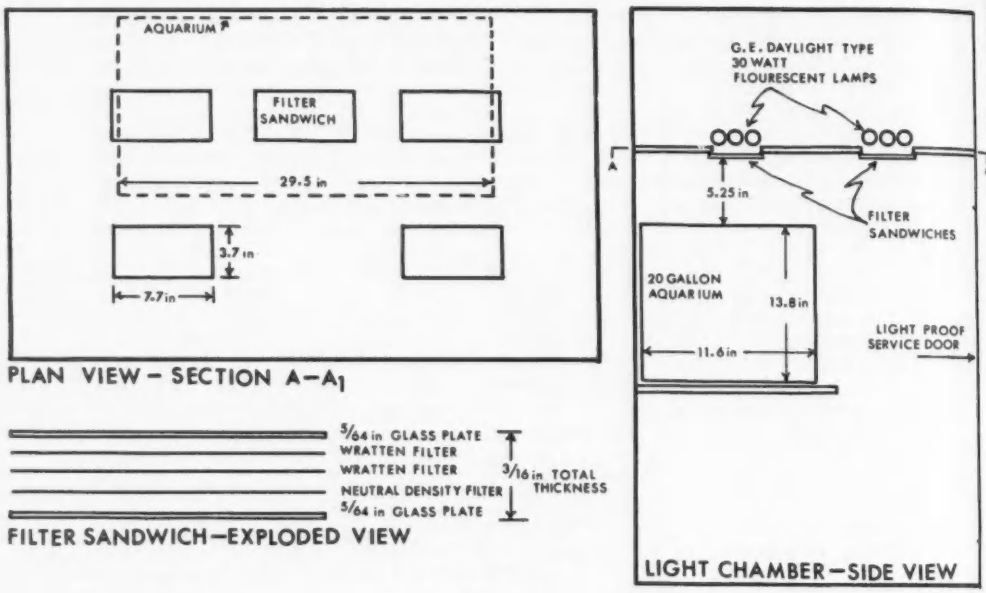


FIG. 3. Experimental light chambers.

are shown in Fig. 2. Only neutral density filters were used in the (experimental) control sandwiches so that the wavelength pattern in this box was simply that of the fluorescent bulbs minus wavelengths shorter than 390 mμ filtered out by the two glass plates.

Photoperiods for both the wild and experimental groups were comparable ranging from 8 hr light in January to 15 hr in June (Fig. 1). In the experimental light boxes photoperiods were controlled by a single photoelectric cell and relay-positioned in an east window.

From the initial collection of 440 fish, 20 were randomly sampled for weight, length, and degree of sexual maturity to provide a base line from which the performance of the experimental groups could be assessed. The remainder were randomly assigned to the five experimental tanks.

Samples of 10 fish were taken from each chamber at intervals of 3–4 weeks. Wild samples of approximately 20 fish were collected within a few days of each experimental sample along with measures of water temperature and salinity.

The sampled fish were anesthetized in 0.7% urethane, weighed (10 mg), standard lengths (snout to last vertebra) were taken, heads removed, bellies opened, and the whole fish fixed in Bouin's fixative (Pantin, 1964). The gonads were removed after 24 hr, weighed separately, and prepared for sectioning according to Pantin (1964). The tissues were embedded in Paraplast wax, sectioned at 10 μ, and stained with Delafield's hematoxylin and eosin (Humason, 1962). Approximately 75 sections were taken from the middle region of each gonad.

In sample 7 and 8, the gonads were weighed fresh before fixing in Bouin's fluid. To convert preserved weights to live weights correction factors were calculated by individually weighing a fresh sample of 20 testes and 20 ovaries and then reweighing each after periods of 24 and 48 hr in Bouin's fixative.

In the first four samples, individual body weights were not associated with gonad weights; thus, it was impossible to identify body weights as male or female. To calculate the ovary:body weight ratios the average female body weight per sample was calculated by finding a relationship between male and female weights from a sexed group of 21 females and 19 males in sample 5.

This provided a conversion factor for a sample composed of equal numbers of males and females. By interpolation, conversion factors for samples 1–4 were calculated depending on male to female ratio. By knowing the average weight of the sample, the number of each sex, and the conversion factor, female averages were obtained.

Histologically the state of ovary development was assessed by establishing criteria of oocyte maturity and defining several stages of sexual development. The criteria and stages defined follow those of Braekevelt and McMillan (1967) with minor modifications. Testes development was treated similarly by defining four stages of spermatogenesis. The criteria established by Ashan and Hoar (1963) were used in describing the state of maturity of each male as inactive, slightly active, moderately active, or fully active.

Analyses of variance and Duncan's multiple range test were used to evaluate the results statistically (Li, 1964; Duncan, 1957).

RESULTS

GROWTH

Growth patterns for the wild and experimental groups are given in Fig. 4 and 5 and Table 1. Comparisons of the various groups show similar growth rates over the full observation period for the wild and five experimental groups.

Certain minor differences are evident. During the period February to April the experimental fish were consistently heavier than the wild fish. This

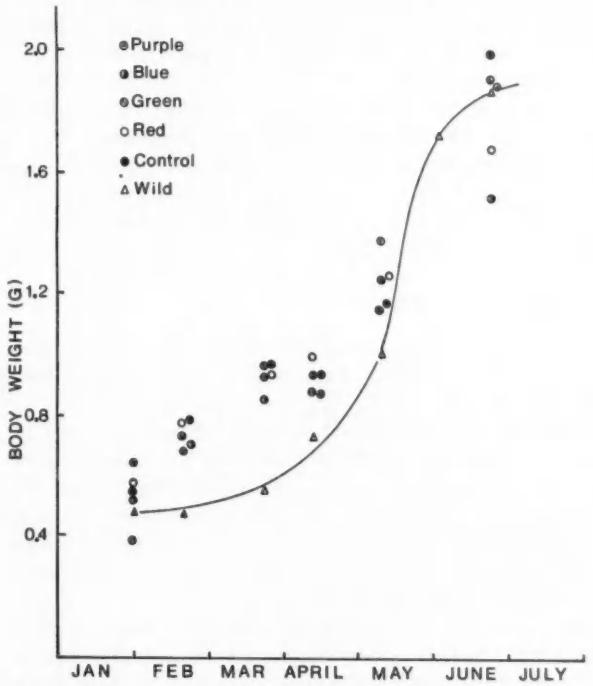


FIG. 4. Seasonal body weight (live) changes (sexes combined).

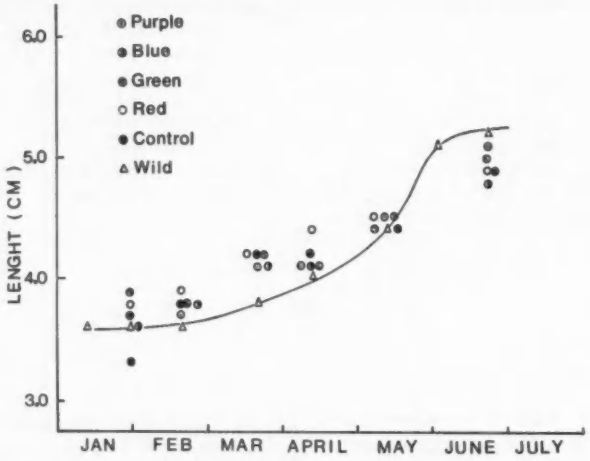


FIG. 5. Seasonal body length (standard) changes (sexes combined).

difference was probably due to the laboratory diet as evidenced by large fat deposits found during dissection. There is also an indication that the experimental fish were slightly longer during the same period. The combination of diet and higher temperature (16 C) may have led to a slightly augmented growth rate in the experimental groups. The June values suggest that the blue and red groups were significantly smaller than the others. However, in the preceding five sampling periods these groups showed no unusual differences so that this is interpreted as a sampling error. The final sample consisted of all of the fish remaining in the tanks including a number of small animals that had escaped the earlier sampling.

SEXUAL MATURATION

Two methods of assessing sexual maturation were employed, gonad weight changes and a histological index consisting of nine stages, seven of which are illustrated in Fig. 6.

Figure 7 shows the individually variable seasonal pattern of maturation in this species. For example, in March and April (samples 5 and 6) when most females were still immature (stage 3) a few (3 out of 66) were already nearing sexual maturity (stage 7). Because of this variability and its effect on the small sample sizes used in this study extreme values (those to the right of the hatched line in Fig. 7) have been omitted from the data shown in Fig. 8 but are included in the values of Table 2.

The seasonal pattern of ovary development assessed histologically is shown in Fig. 8 and Table 2. No differences were observed between the various experimental groups exposed to different wavelengths. Also the experimental and wild groups showed similar rates of development except in late June

TABLE 1. Body weights (g, fresh) and lengths (cm, standard), sexes combined. Numbers in parentheses are sample sizes.

Sample			Groups						F values[a]	Ranked means[b]
Date (*1969*)	No.		Wild	Purple	Blue	Green	Red	Control		
Jan. 2	1	Wt	0.3906(20)							
		Length	3.6							
Jan. 30	2	Wt	0.4597± 0.142(20)[c]	0.3794± 0.0108(11)	0.5141± 0.089(610)	0.6418± 0.0610(10)	0.5762± 0.0322(10)	0.5455± 0.0157(10)	2.8108	PWBCRG
		Length	3.6± 0.0918(20)	3.3± 0.0862(11)	3.6± 0.4360(10)	3.9± 0.3337(10)	3.8± 0.1756(10)	3.7± 0.0826(10)	2.9923	PWBCRG
Feb. 20	3	Wt	0.4637± 0.0142(19)	0.6850± 0.0644(10)	0.7038± 0.0405(10)	0.7382± 0.0194(10)	0.7696± 0.0316(10)	0.7065± 0.0530(11)	5.6023	WPBCGR
		Length	3.6± 0.0923(19)	3.7± 0.1765(10)	3.8± 0.1223(10)	3.8 ± 0.0511(10)	3.9± 0.1160(10)	3.8± 0.2020(10)		
Mar. 20–25	4	Wt	0.5406(23)	0.9347(10)	0.8666(10)	0.9698(10)	0.9407(10)	0.9769(10)		
		Length	3.8(23)	4.1(10)	4.1(10)	4.2(10)	4.2(10)	4.2(10)		
Apr. 10–14	5	Wt	0.7353(8)	0.8876(10)	0.9466(10)	0.8818(10)	1.0364(10)	0.9470(10)		
		Length	4.0(8)	4.1(10)	4.1(10)	4.1(10)	4.4(10)	4.2(10)		
May 6–12	6	Wt	1.0067± 0.0953(10)	1.3797± 0.3840(10)	1.2500± 0.2091(10)	1.1593± 0.0465(10)	1.2661± 0.1420(10)	1.1758± 0.1057(10)		
		Length	4.4± 0.1632(10)	4.5± 0.2995(10)	4.5± 0.2476(10)	4.4± 0.0667(10)	4.5± 0.2155(10)	4.4± 0.1645(10)		
June 2	7	Wt	1.7190(10)							
		Length	5.1(10)							
June 19–24	8	Wt	1.8595± 0.0866(19)	1.8969± 0.1400(26)	1.5119± 0.2157(31)	1.9034± 0.1512(25)	1.69611± 0.1389(25)	1.9880± 0.3916(26)	4.4824	BRWPGC
		Length	5.2± 0.0332(19)	5.1± 0.1562(26)	4.8± 0.1757(31)	5.0± 0.0849(25)	4.9± 0.1112(25)	4.9± 0.1491(26)	4.5882	BRGPCW

[a]Only values at or above the 0.05 level of significance are shown.
[b]Letters refer to the first letter of each group (B = Blue etc.).
[c]Standard deviation.

when the wild and blue groups showed a significant difference. The slightly lower average maturity of the blue group is due to the presence of a few, small, immature individuals included in the final sample as noted above.

Ovary weight changes during the same period are shown in Fig. 9 as an ovary–body weight ratio and in Table 3. Once again all groups showed similar seasonal ovary weight changes with the blue group lagging in the final sample for the reasons discussed above.

A comparison of the methods of assessment (ovary weight vs. histological index) shown in Fig. 10 yielded a correlation coefficient, R = 0.974. Visual inspection of Fig. 9 and 10 indicates that during the period of January to April when the ovary weights showed little change the histological index showed a change from stage III to stage IV (development of the peripheral yolk vacuoles), so that during this period the histological index provided a more sensitive indication of egg maturation. Beyond April the marked changes in gonad weight correlated with the further development of primary yolk vacuoles and the elaboration of secondary yolk (Fig. 6, stages V–VIII).

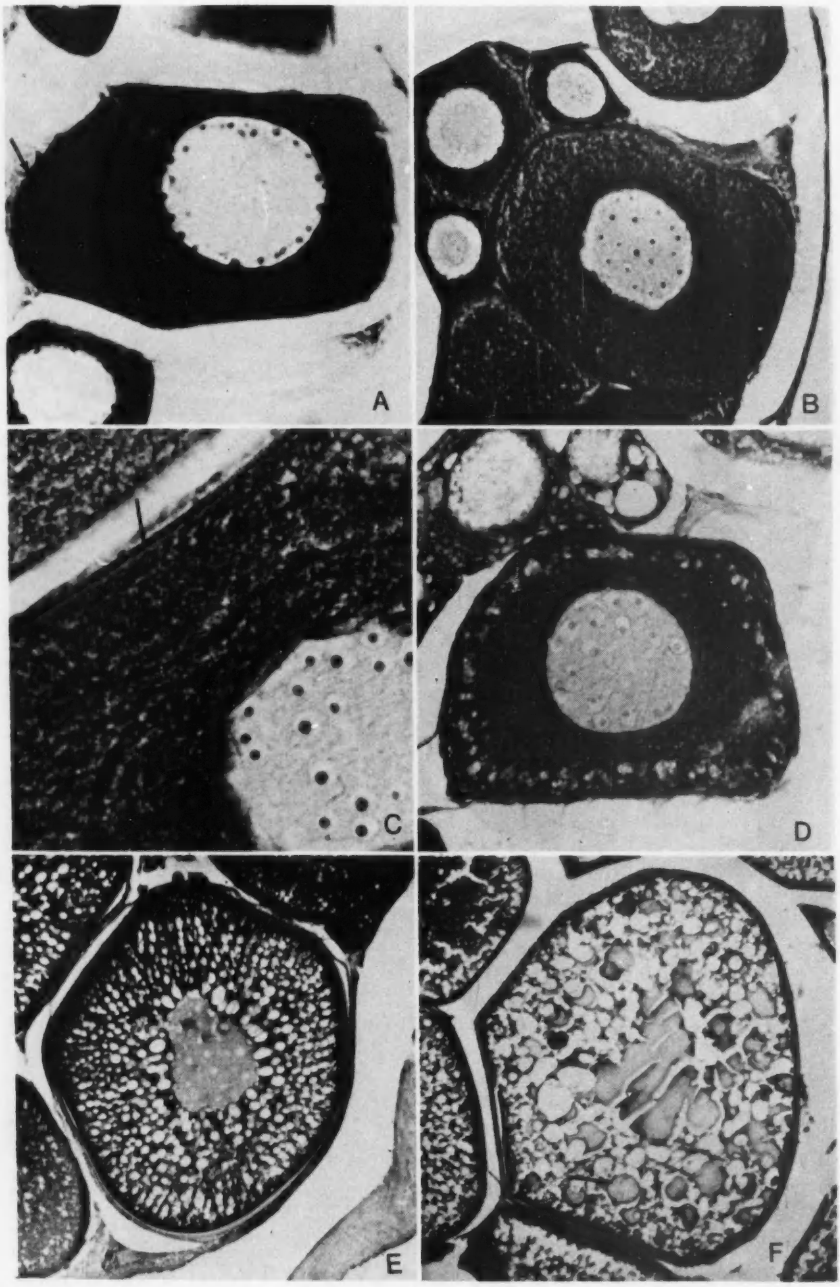
Male maturation was assessed by means of a histological index described by Ashan and Hoar (1963) for this species. By late June all experimental males were classed as fully active as evidenced by seminiferous tubules packed with sperm and spermatids and the development of mature male colouration. The seasonal pattern of development is shown for the five light treatments in Fig. 11. Information for the wild fish (Table 2) indicating an initial (January–April) decline in maturity suggests that the wild group was probably sampled from a seasonally changing rather than resident population.

DISCUSSION

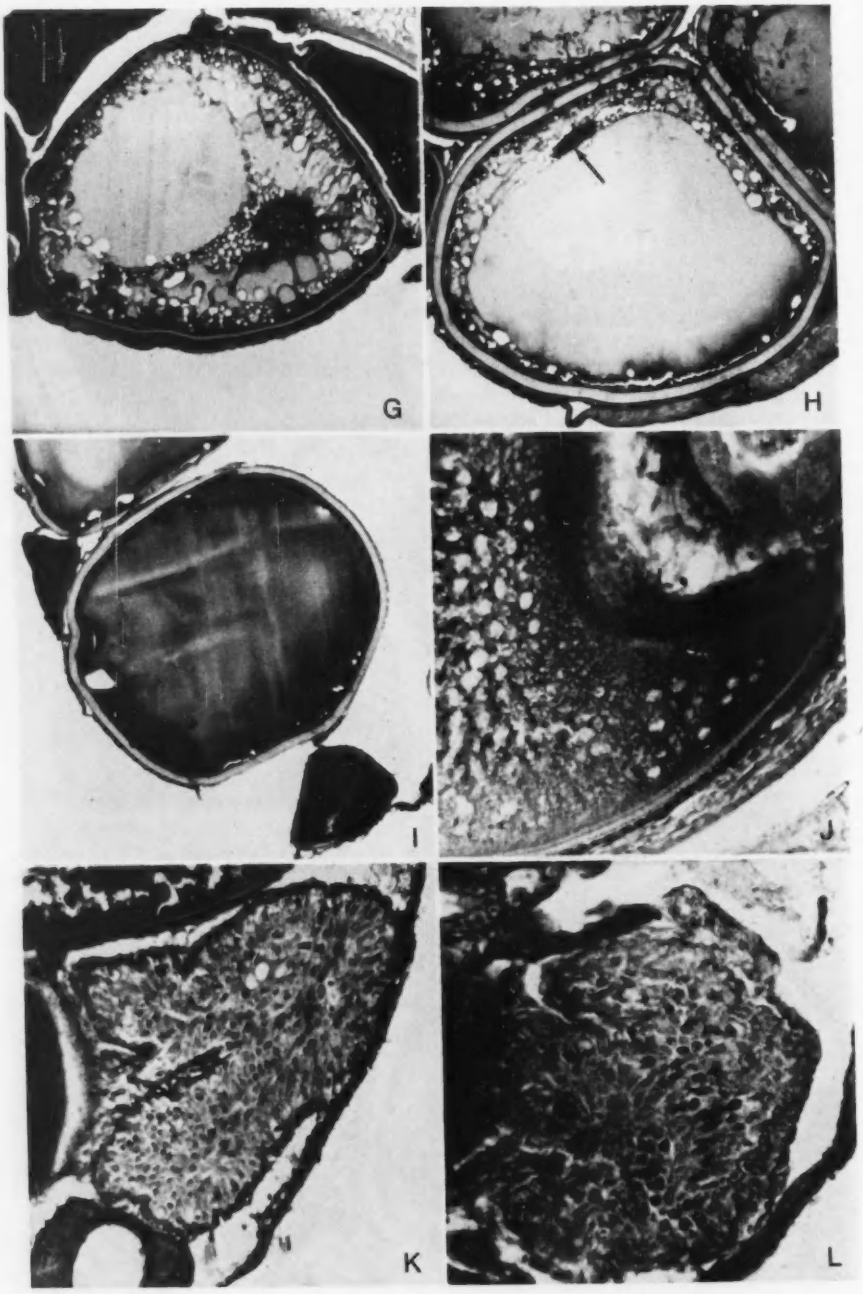
The primary purpose of this experiment was to test the effects of light quality on the photoperiod mechanism controlling sexual maturation in the threespine stickleback. The results demonstrate that visible light ranging from 388 to 653 mμ (long ultraviolet to short red) provides an effective stimulus for activating this seasonal timing mechanism.

This contrasts sharply with work on ducks where Benoit et al. (1952) have shown that the action spectrum of the photoperiod mechanism has its

FIG. 6. Photomicrographs of frontal sections from the midsection of *Gasterosteus aculeatus* ovaries sectioned at 10 μ and stained with Delafield's hematoxylin and eosin. (A) Intermediate between stage II and III vitelline membrane beginning to form (arrow) ×400. (B) Stage III vitelline membrane clearly defined. ×200. (C) Edge of stage II and III oocytes showing presence and absence of vitelline membrane (arrow). ×500. (D) Stage IV oocyte with peripheral primary yolk vacuoles. ×312. (E) Stage V oocyte with primary yolk vacuoles filling cytoplasm. ×125. (F) Stage VI oocyte showing globules of secondary yolk (arrow). ×101. (G) Early stage VII oocyte with secondary yolk globules merging. Note shifting position of nucleus. ×101. (H) Late stage VII oocyte with secondary yolk almost filling cell, nucleus has become flattened and moved towards periphery. ×79. (I) Stage VIII. Oocyte is filled with yolk. At this time spawning occurs. (J) Early stage of hypertrophic atresia. Note enlarged follicular cells and erosion of vitelline membrane (arrows). ×312. (K) Later stage of atresia with loose mass of follicular cells filling oocyte. ×200. (L) After hypertrophic atresia the remaining mass of cells collapses to form a tight ball. ×312.



McInerney and Evans — J. Fish. Res. Bd. Canada



McInerney and Evans — J. Fish. Res. Bd. Canada

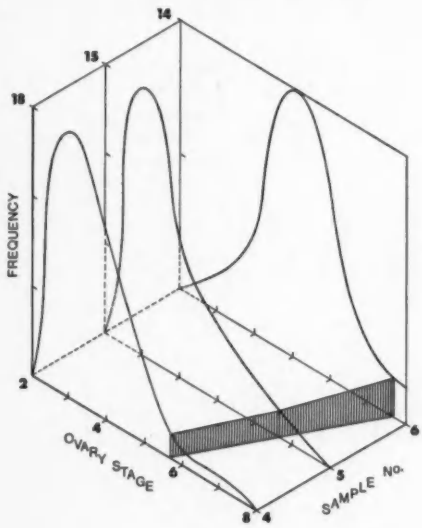


FIG. 7. Variable ovary stage (histological index) development for samples 4 (March 20–25), 5 (April 10–14), and 6 (May 6–12). Values to the right of the hatched area are excluded from Fig. 8. See text for explanation.

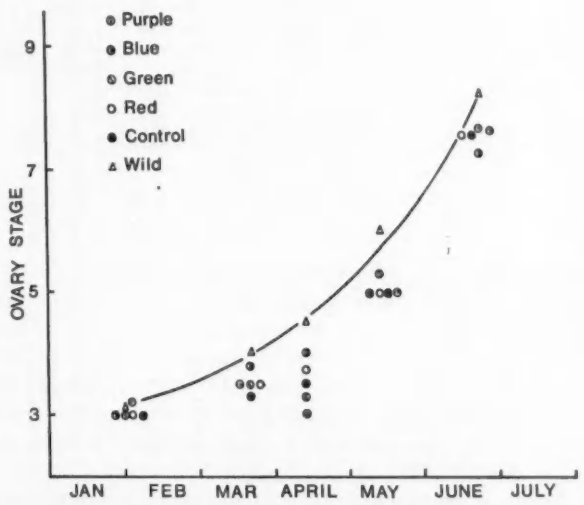


FIG. 8. Seasonal changes in ovary development. Refer to Fig. 6 for an explanation of ovary stages

maximum generally in the wavelength band from 600 to 750 mμ. He also showed that light receptors capable of mediating the photoperiod response are located both in the retina and in the brain (hypothalamus and rhiencephalon) and that the latter are sensitive to almost the entire visible spectrum. However, these latter receptors are not apparently normally susceptible to

TABLE 2. Histological index, male and female gonads.[a] Numbers in parentheses are sample sizes.

Sample			Groups						F values[b]	Ranked means[c]
Date (*1969*)	No.		Wild	Purple	Blue	Green	Red	Control		
Jan. 2	1	F[d]	3.5(11)							
		M	2.5(5)							
Jan. 30	2	F	3.2(13)	3.2(6)	3.0(4)	3.0(4)	3.0(4)	3.0(5)		
		M	2.4(4)	1.3(3)	2.7(3)	2.5(6)	2.2(5)	2.2(5)		
Mar. 20–25	4	F	4.0(12)	4.0(5)	3.8(5)	4.2(5)	3.5(6)	3.3(6)		
		M	2.0(8)	2.7(3)	2.8(4)	2.5(4)	3.0(3)	2.5(4)		
Apr. 10–14	5	F	5.3(3)	3.0(4)	4.0(4)	3.3(6)	3.8(4)	3.5(6)		
		M	1.5(6)	3.0(6)	3.4(5)	3.3(4)	2.8(6)	3.0(4)		
May 6–12	6	F	6.0± 0.500[e](5)	5.6± 4.300(5)	5.0± 2.000(5)	5.3± 0.267(6)	5.4± 1.620(7)	5.0± 0.750(9)		
		M	2.6(5)	3.8(3)	3.2(5)	3.3(4)	4.0(3)	4.0(1)		
June 19–25	8	F	8.3± 0.667(15)	7.8± 0.618(18)	7.3± 1.163(16)	7.7± 0.363(16)	7.6± 0.5428(15)	7.6± 0.543(15)	2.7591	BRCGPW
		M	–	4.0(6)	4.0(9)	4.0(5)	4.0(6)	4.0(6)		

[a]Refer to Fig. 6 for ovary index and to Materials and Methods for testis index.
[b]Only values at or above the 0.05 level of significance are shown.
[c]Letters refer to the first letter of each group (B = Blue etc.).
[d]F = female; M = male.
[e]Standard deviation.

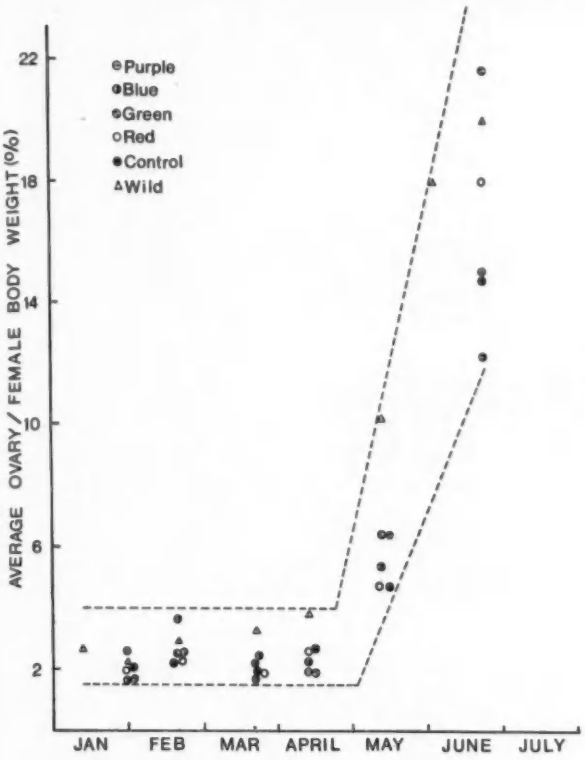


FIG. 9. Seasonal changes in ovary-body weight ratios (both fresh weights).

stimulation by environmental light in intact animals. It is possible though unlikely because of the heavy skull structure, that the broad response spectrum in the present study is due to stimulation of encephalic receptors. Another interpretation for the wide action spectrum is that fish show a primitive pattern of retinal connections with most optic fibers passing to the thalamus and optic lobe regions of the brain. Birds along with other terrestrial vertebrate groups have undergone a changing pattern of retinal connections with many fibers passing indirectly to the forebrain (Polyak, 1957). Possibly this evolutionary rewiring has included the loss of certain shorter wavelength receptors mediating the photoperiod response, though comparative information is so scarce in this area that any conclusion must be highly speculative.

The sensitivity of the sticklebacks photoperiod transducer(s), which includes the shorter blue wavelengths, is consistent with the spectral transmission properties of the aquatic environment where the longer red wavelengths are rapidly absorbed near the surface (Sverdrup et al., 1942). It also parallels the spectral sensitivity pattern inducing insect diapause where the effective wavelengths lie between 400 and 500 mμ (Beck, 1968).

TABLE 3. Female body and ovary weights (g, fresh). Numbers in parentheses are sample sizes.

Sample			Groups						F values[a]	Ranked means[b]
Date (*1969*)	No.		Wild	Purple	Blue	Green	Red	Control		
Jan. 2	1	O[c]	0.0102(15)							
		B	0.3749(15)							
Jan. 30	2	O	0.0090± 0.0374[d](13)	0.0088± 0.1052(6)	0.0072± 0.0767(6)	0.0088± 0.1947(4)	0.0094± 0.0729(4)	0.0096± 0.0329(5)		
		B	0.4102(13)	0.3331	0.4588(6)	0.5382(4)	0.4832(4)	0.4721(5)		
Feb. 20	3	O	0.0120± 0.0904(9)	0.0154± 0.1725(6)	0.0212± 0.5502(4)	0.0150± 0.1061(6)	0.0174± 0.1705(9)	0.0150± 0.2207(6)		
		B	0.4097(9)	0.6113(6)	0.5902(4)	0.6389(6)	0.7489(9)	0.6819(6)		
Mar. 20–25	4	O	0.0154(12)	0.0256(5)	0.0180(5)	0.0332(6)	0.0162(7)	0.0168(6)		
		B	0.4679(12)	0.8090(5)	0.7500(5)	0.8639(6)	0.8648(7)	0.8718(6)		
Apr. 10–14	5	O	0.0626(3)	0.0142(4)	0.0174(5)	0.0144(6)	0.0264(4)	0.0200(6)		
		B	0.6166(3)	0.7443(4)	0.7793(5)	0.7641(6)	1.0373(4)	0.7812(6)		
May 6–12	6	O	0.0762± 0.0012(5)	0.1574± 0.0407(6)	0.0662± 0.0032(5)	0.0746± 0.0027(6)	0.1144± 0.0217(7)	0.0546± 0.0023(9)		
		B	0.7443(5)	1.3797(6)	1.2501(5)	1.1593(6)	1.2661(7)	1.1758(9)		
June 2	7	C	0.2886(8)							
		B	1.7254(8)							
June 19–25	8	O	0.3783± 0.0462(15)	0.2903± 0.0318(20)	0.1909± 0.0264(18)	0.4217± 0.0666(17)	0.3053± 0.0278(17)	0.3194± 0.0616(17)	2.5443	BPRCWG
		B	1.8873± 0.986(16)	1.9263± 0.1619(20)	1.5654± 0.2954(18)	1.9469± 0.1826(17)	1.7003± 0.1771(17)	2.1648± 0.4618(17)	3.2937	BRWPGC

[a]Only values at or above the 0.05 level of significance are shown.
[b]Letters refer to the first letter of each group (B = Blue etc.).
[c]O = ovary; B = body.
[d]Standard deviation.

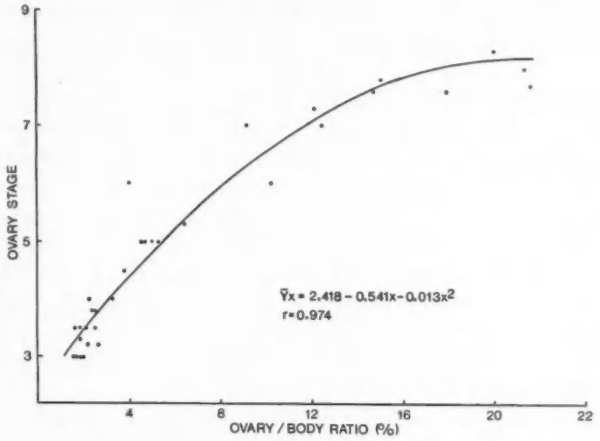


FIG. 10. Correlation of ovary–body weight ratios (gonad weight index) with ovary stage changes (histological index).

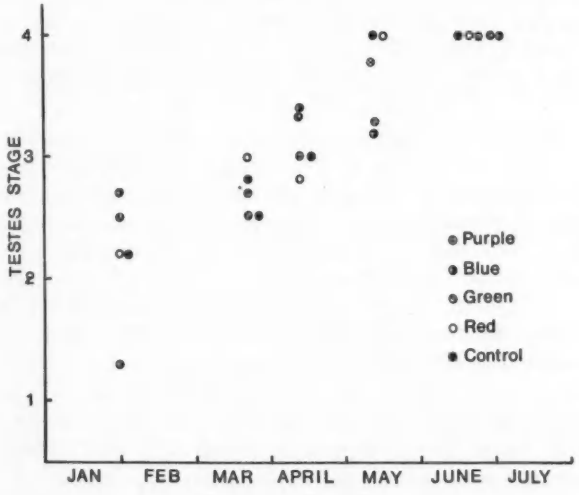


FIG. 11. Seasonal changes in testes stage (histological index, Ashan and Hoar, 1963).

Several other results of this study merit discussion. Measurements of light "intensities" in photoperiod studies are usually given in lux (lumens/m^2), a unit based on the spectral sensitivity of the human retina and therefore of questionable value in a comparative context. Continued use of such units where the action spectrum of the photoperiod mechanism has been clearly shown to differ from the human eye (Benoit et al., 1952; Beck, 1968) is regretable. In the present study the levels of light energy reaching the fish were

relatively low, less than 0.37×10^3 erqs/cm^2-sec (0.037 mw/cm^2). This is equivalent to an illuminance level of approximately 87 lux (red box), 230 lux (green), 41 lux (blue,) and 5 lux (purple) (Billings, 1957). Beck (1968), in reviewing the insect literature, and Farner (1961), in his review of vertebrates, state that minimum threshold "intensities" for photoperiod induction in both groups appear to be about 1 lux per figure, not appreciably different from the value for the purple box in this study.

Baggermann (1957) in studying the effects of light "intensity" found that threespine sticklebacks exposed to 269–323 lux (25–30 ft-c) matured slightly more rapidly, 28 days, than those exposed to 161 lux (15 ft-c), 32 days. The fact that maturation rates comparable to those in wild fish were obtained at illuminance levels of less than 5 lux (at 420 mμ) in the present study demonstrates a sensitivity for the photoperiod mechanism comparable to those of insects and birds as noted above.

Comparison of the maturation pattern of the threespine stickleback in this study with that of the brook stickleback (Braekevelt and McMillan, 1967) shows certain differences. Although the gonad–body weight ratios in January are comparable (about 2%), at maturity they are distinctly different, approximately 12% for the brook versus 27% for the threespine stickleback. This is probably accounted for by a difference in egg size rather than fecundity: threespine stickleback, 1.44–1.52 mm (van den Assem, 1967) versus brook stickleback, approximately 1 mm (Winn, 1960; Braekevelt and McMillan, 1967).

Histological comparisons reveal similar patterns of cytological development (including hypertrophic atresia, Fig. 6, J–L) except for the final stage of maturation. Whereas mature brook stickleback eggs retain a distinct peripheral rim of cytoplasm and the secondary yolk shows a globular texture (Braekevelt and McMillan, 1967, fig. 20 and 36,) in the threespine stickleback the cytoplasmic rim appears to be relatively much thinner and the secondary yolk forms a uniform untextured mass (Fig. 6I). This additional step in yolk differentiation has been labelled stage VIII for the threespine and probably accounts for the larger size of the threespine egg noted above.

From a practical, standpoint the results demonstrate that for experimental photoperiod studies under laboratory conditions any common light source (including most incandescent and fluorescent types) can be expected to elicit normal photoperiod responses. It also shows, assuming sticklebacks to be representative of other fishes, that the use of dim red or blue lights for behavioural observations might be expected to affect photoperiod induction if used during the dark period. Dim red lights are sometimes used for behavioural observations of insects on the mistaken premise that insects are insensitive to red light (Beck, 1968).

ACKNOWLEDGMENTS

Financial support for this study was provided by N.R.C. Operating Grant A-2069.

REFERENCES

ASHAN, S. N., AND W. S. HOAR. 1963. Some effects of gonadotropic hormones on the threespine stickleback, *Gasterosteus aculeatus*. Can. J. Zool. 41: 1045–1053.

ASSEM, J. VAN DEN. 1967. Territory in the threespine stickleback, *Gasterosteus aculeatus* L. A study in intraspecific competition during reproduction. Behaviour (Suppl.) 16: 164 p.

BAGGERMAN, B. 1957. An experimental study on the timing of breeding and migration in the three-spined stickleback (*Gasterosteus aculeatus* L.). Arch. Neerlandaises Zool. 12: 105–318.

BECK, S. D. 1968. Insect photoperiodism. Academic Press, Inc., New York. 288 p.

BENOIT, J., I. ASSENMACHER, AND F. X. WALTER. 1952. Différences de sensibilité de la rétine du canard aux radiations colorées dans le réflexe pupillaire et dans le réflexe opto-sexuel. C. R. Seances 146: 1027–1030.

BILLINGS, B. H. 1957. Optics, Sect. 6–9. *In* D. E. Gray [ed.] American Institute of Physics Handbook. McGraw-Hill, Book Co., Inc., New York.

BRAEKEVELT, C. R., AND D. B. McMILLAN. 1967. Cyclic changes in the ovary of the brook stickleback, *Eucalia inconstans* (Kirtland). J. Morphol. 123: 373–396.

CRESCITELLI, F. 1958. The natural history of visual pigments, p. 30–51. *In* R. W. Newburgh [ed.] Photobiology. Proceedings of the Nineteenth Annual Biology Colloquium, Oregon State College, Corvallis, Oreg.

DUNCAN, D. B. 1957. Multiple range tests for correlated and heteroscedastic means. Biometrics 13: 164–176.

FARNER, D. S. 1958. Photoperiodism in animals with special reference to avian testicular cycles, p. 17–29. *In* R. W. Newburgh [ed.] Photobiology. Proceedings of the Nineteenth Annual Biology Colloquium, Oregon State College, Corvallis, Oreg.
1961. Comparative physiology: photoperiodicity. Ann. Rev. Physiol. 23: 71–96.

HARRINGTON, R. W., JR. 1959. Photoperiodism in fishes in relation to the annual cycle, p. 651–667. *In* R. B. Withrow [ed.] Photoperiodism and related phenomena in plants and animals. American Association for the Advancement of Science, Washington, D.C.

HUMASON, G. L. 1962. Animal tissue techniques. W. H. Freeman and Co., San Francisco, Calif. 569 p.

LI, J. C. R. 1964. Statistical inference I. Edward Brothers, Ann Arbor, Mich. 658 p.

McINERNEY, J. E. 1969. Reproductive behaviour of the blackspotted stickleback, *Gasterosteus wheatlandi*. J. Fish. Res. Bd. Canada 26: 2061–2075.

PANTIN, C. F. A. 1964. Notes on microscopical technique for zoologists. Cambridge University Press, London. 77 p.

POLYAK, S. 1957. The vertebrate visual system. Univ. of Chicago Press, Chicago, Ill. 1390 p.

SVERDRUP, H. V., M. W. JOHNSON, AND R. E. FLEMING. 1942. The oceans. Prentice-Hall, Englewood, New Jersey. 1087 p.

TINBERGEN, N. 1951. The study of instinct. Clarendon Press, Oxford. 228 p.

WINN, H. E. 1960. Biology of the brook stickleback. Amer. Midland Natur. 63: 424–438.

WOLFSON, A. 1964. Animal photoperiodism, p. 1–49. *In* A. C. Giese [ed.] Photophysiology, Vol. I. Academic Press, Inc., New York.

Analysis of Nonlinear Models — the Nonlinear Response Surface

J. K. LINDSEY,[1] D. F. ALDERDICE, AND L. V. PIENAAR[2]

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

LINDSEY, J. K., D. F. ALDERDICE, AND L. V. PIENAAR. 1970. Analysis of nonlinear models — the nonlinear response surface. J. Fish. Res. Bd. Canada 27: 765–791.

The role of the likelihood function in statistical inference is discussed, with particular reference to problems where exact probability (structural or fiducial) distributions for the parameters are unknown. The methods and uses of response surface methodology are briefly discussed, especially the usefulness of nonlinear models, and the difficulties in inferences about these models. Likelihood inference is suggested as a method of overcoming these problems, and the procedures for this are developed. Three examples using biological data demonstrate the procedures.

Received August 25, 1969

INTRODUCTION

PROGRESS IN INFERENCE PROCEDURES involving nonlinear models has been slow because the calculation of any type of probability interval (confidence, significance, or fiducial) for the parameters has proven to be difficult or impossible. Draper and Smith (1966) propose to calculate approximate 100 $(1 - \alpha)\%$ confidence regions for the parameters of nonlinear models in the same manner as exact regions are determined in linear models, resulting in exact confidence regions, but with unknown probability levels. These regions can also be considered as exact 100 $(1 - \alpha)\%$ significance regions (see the discussion of significance intervals by Sprott, MS, 1968). Since no sufficient estimates for the parameters exist in nonlinear models, no fiducial distributions of the parameters can be calculated, and hence no fiducial intervals.

Fisher (1956) and more recently Barnard et al. (1962) have maintained that likelihoods rather than probabilities are relevant when dealing with experimental results. The likelihood function is simply the probability function with the observed points in the sample space being considered as known constants, and the parameters as unknown. Maximum likelihood estimates of the parameters are the coordinates of that point in the parameter space that make the likelihood function largest. The relative likelihood of any other value (point in the parameter space) is the ratio of the likelihood function, with these parameter values substituted into it, to the likelihood function with the maximum likelihood estimates inserted, and is always less than one. Thus,

---

[1]Present address: Imperial College, University of London, London, England.
[2]Present address (L.V.P.): Washington State University, Pullman, Wash., USA.

Printed in Canada (J1574)

the maximum likelihood estimates may produce a likelihood that is, say, 40 times as large as for some other point in the parameter space (relative likelihood, R = 0.025) and this point is not a likely estimate of the true parameter values. It may readily be seen that the confidence regions given by Draper and Smith (1966, p. 283) are also likelihood regions, and the relative likelihood of any point within the region is greater than some calculable constant (see Beale, 1960; Box and Hunter, 1965).

The relative likelihood may be compared logically with a significance test. A significance test yields the total probability, given the hypothesis, of observing any set of data at least as improbable as that observed. A relative likelihood gives the relative probability as a ratio of the probability, given the hypothesis, of observing the given set of data to the probability of observing the data given the hypothesis making the observations most probable under the assumptions of the model. Hence, the relative likelihood does not involve consideration of the probability of observing data sets not actually observed. As well, the likelihood uses all of the information supplied by the data, whereas the significance test may not.

When the parameter space is multidimensional, the maximized relative likelihood is a useful statistic for summarizing information about one of the parameters in the space (see Sprott and Kalbfleisch, 1969). To calculate the relative likelihood of some value of one parameter in such a space, this parameter is held at the specified value and the likelihood function maximized over all of the other parameters in the space to give a maximized relative likelihood. In this manner, a maximized relative likelihood graph may be plotted for each of the parameters in the parameter space, each graph reaching a relative likelihood of unity at the maximum likelihood estimates. If the parameter estimates are independent, they will remain at their maximum likelihood values for all chosen values of any one of them.

As will be shown, the likelihood approach to statistical inference provides a satisfactory method of coping with nonlinear models. Likelihood regions can be calculated for any estimable parameter or combination of parameters in the model under consideration, provided that some probability distribution is assumed before the experiment is performed.

NOTATION

Represent n observations in a $(m + 1)$-dimensional *sample space* as

$$(y_i, x_{i1}, x_{i2}, \ldots, x_{im}) \quad i = 1, \ldots, n. \tag{1}$$

In response surface models, y_i is the *response* determined by the point, $\mathbf{x}_i = (x_{i1}, x_{i2}, \ldots, x_{im})$ in the m-dimensional *factor space*, according to the model.

The general form of the *model* is

$$y_i = f(\mathbf{x}_i, \boldsymbol{\theta}) + \epsilon_i \tag{2}$$

where $\boldsymbol{\theta} = (\theta_1, \theta_2, \ldots, \theta_p)$ is a point in the p-dimensional *parameter space*. In

this paper the responses will be assumed to be independently normally distributed with homogeneous variance and with mean $f(\mathbf{x}_i, \boldsymbol{\theta})$:

$$y_i \sim \mathrm{N}[f(\mathbf{x}_i,\boldsymbol{\theta}),\sigma^2]. \tag{3}$$

Thus, the probability distribution of the responses is given by

$$g(y_i,\mathbf{x}_i;\boldsymbol{\theta}) = (2\pi\sigma^2)^{-n/2} \exp-\left\{\frac{1}{2\sigma^2}\sum_i [y_i - f(\mathbf{x}_i,\boldsymbol{\theta})]^2\right\}. \tag{4}$$

Since $\hat{\sigma}^2 = \Sigma[y_i - f(\mathbf{x}_i, \hat{\boldsymbol{\theta}})]^2/n$ is the maximum likelihood estimate of the variance, the *likelihood function*, maximized with respect to σ^2, is

$$\mathrm{L}(\boldsymbol{\theta}) = c\left\{\Sigma [y_i - f(\mathbf{x}_i,\boldsymbol{\theta})]^2\right\}^{-n/2} \tag{5}$$

where $c = (2\pi\mathrm{e}/n)^{-n/2}$ is a constant. The *maximum likelihood estimates* of the parameters will be denoted by $\hat{\boldsymbol{\theta}}$; obtained by maximizing $\mathrm{L}(\boldsymbol{\theta})$, they are identical to the least squares estimates obtained by minimizing $\Sigma_i[y_i - f(\mathbf{x}_i,\boldsymbol{\theta})]^2$ under the above specified distributional assumptions about the responses.

The *relative likelihood* of any point $\boldsymbol{\theta}$ in the parameter space is given by

$$\mathrm{R}(\boldsymbol{\theta}) = \mathrm{L}(\boldsymbol{\theta})/\mathrm{L}(\hat{\boldsymbol{\theta}}). \tag{6}$$

If a transformation of the response of the form $y^{(\gamma)}$ is considered, where $\boldsymbol{\gamma}$ is a vector of parameters, e.g., $\boldsymbol{\gamma} = (\gamma_1, \gamma_2)$ with $y^{(\gamma)} = (y + \gamma_1)^{\gamma_2}$, then the distribution of the responses becomes

$$g'(y_i,\mathbf{x}_i;\boldsymbol{\gamma},\boldsymbol{\theta}) = (2\pi\sigma^2)^{-n/2} \exp-\left\{\frac{1}{2\sigma^2}\Sigma_i[y_i^{(\gamma)} - f(\mathbf{x}_i,\boldsymbol{\theta})]^2\right\}\mathrm{J}(y,\boldsymbol{\gamma}) \tag{7}$$

where $\mathrm{J}(y,\boldsymbol{\gamma})$ is the Jacobian of the transformation. The likelihood function then becomes

$$\mathrm{L}(\boldsymbol{\gamma},\boldsymbol{\theta}) = c\left\{\Sigma_i[y_i^{(\gamma)} - f(\mathbf{x}_i,\boldsymbol{\theta})]^2\right\}^{-n/2} \mathrm{J}(y, \boldsymbol{\gamma}) \tag{8}$$

and the relative likelihood

$$\mathrm{R}(\boldsymbol{\gamma},\boldsymbol{\theta}) = \mathrm{L}(\boldsymbol{\gamma}, \boldsymbol{\theta})/\mathrm{L}(\hat{\boldsymbol{\gamma}},\hat{\boldsymbol{\theta}}). \tag{9}$$

In the remainder of the paper, a nonlinear response surface will be considered as a specific example of the general nonlinear model. The nonlinear response surface model chosen has the form

$$y_i^{\gamma} = \beta_o + \sum_{j=1}^{m} \beta_j x_{ij}^{\alpha_j} + \sum_{j=1}^{m}\sum_{k=j}^{m} \beta_{jk} x_{ij}^{\alpha_j} x_{ik}^{\alpha_k} + \epsilon_i \tag{10}$$

where the response y_i is raised to the power γ, and each coordinate x_{ij} of the point $\mathbf{x}_i$ in the factor space, to the power α_j. Thus,

$$y_i^{(\gamma)} = y_i^{\gamma}$$

$$\theta = (\gamma,\beta_o,\beta_1,\ldots,\beta_m,\beta_{11},\beta_{12},\ldots,\beta_{mm},\alpha_1,\ldots,\alpha_m). \tag{11}$$

The Jacobian becomes

$$J(y,\gamma) = \gamma^n \prod_{i=1}^{n} y_i^{\gamma-1} \tag{12}$$

so that the likelihood function becomes

$$L(\gamma,\beta,\alpha) = \left\{\sum_i [y_i^{\gamma} - \beta_o - \sum_{j=1}^{m} \beta_j x_{ij}^{\alpha_j} - \sum_{j=1}^{m}\sum_{k=j}^{m} \beta_{jk} x_{ij}^{\alpha_j} x_{ik}^{\alpha_k}]^2\right\}^{-n/2} \gamma^n \prod_{i=1}^{n} y_i^{\gamma-1}. \tag{13}$$

The rational for choosing power parameters in the nonlinear response surface model is the great flexibility of such parameters, and the convenience of interpretation; for example, as $\gamma \to 0$, the parameter becomes logarithmic, and as $\gamma \to \pm\infty$, it is exponential.

In most of the discussion the two-dimensional factor space will be considered, i.e., the model

$$y_i^{\gamma} = \beta_o + \beta_1 x_1^{\alpha_1} + \beta_2 x_2^{\alpha_2} + \beta_{11} x_1^{2\alpha_1} + \beta_{22} x_2^{2\alpha_2} + \beta_{12} x_1^{\alpha_1} x_2^{\alpha_2} + \epsilon_i. \tag{14}$$

Examples are given to illustrate the computational procedures in the cases of both two- and three-dimensional factor spaces.

RESPONSE SURFACES

Box and Wilson (1951) originally introduced response surface methodology as a procedure for determining the combination of levels of various factors that produce the optimum response. Efficient methods have been developed for determining this optimum (e.g., method of steepest ascent) from experimental results, as well as for devising efficient experimental designs to gain the maximum information from a given number of points in the factor space (e.g., composite and rotatable designs). More recently, transformations of the factor variables have been suggested (Box and Tidwell, 1962) as a further means of increasing efficiency in the determination of optimum response. In a further step forward, Box and Cox (1964) proposed a procedure for estimating transformation parameters applied to the response variable that would allow the model better to fulfill the assumptions of the probability distribution (see also Dolby, 1963; Draper and Hunter, 1969).

The introduction of transformations not only has allowed more efficient estimation of the optimum of a response surface, *but also has provided an effective means of determining the shape of the response surface in the region of the factor space from which the sample points are taken.* In other words, not only may the transformations be viewed as alterations in the units of measurement of the various factors and the response, but also the complete transformed model may be considered as a nonlinear model describing the observed

data. In the first case, the experimenter is concerned with readily interpretable transformations (e.g., inverse, square root) that still allow the model to explain the observed responses well, i.e., those having high relative likelihood. In the second case, the experimenter accepts the maximum likelihood estimates as the parameters to be used in the model, given the observed data, but wishes to have a measure of the precision of the estimates and of the effect of various other values of the parameters on the model (i.e., the likelihood regions and maximized relative likelihoods). From this, the experimenter may be able to go on to develop a better model, which may be tested with further experiments.

The dual functions performed by transformations apply to both those of the response and those of the coordinates of points in the factor space. In addition, the transformation of the response variable may also bring the model into better conformity with the assumptions of the probability distribution: (i) normality, and (ii) constancy of error variance. Draper and Hunter (1969) demonstrated that the transformation calculated by maximum likelihood estimation, as described by Box and Cox (1964), serves as a type of average in performing these two functions.

In summary, transformations can (i) provide useful insight as to different units of measurement for the variables to be used with a linear model; (ii) provide a more accurate (than the linear model) representation of the relationship under study by means of a nonlinear model, and perhaps lead to a refinement of the model; and (iii) allow the response to fulfill more closely the probability assumptions of the model.

Response surface procedures outlined in most papers use the coded values of the experimental design, with the centre of the design being at the origin in these units. In the analysis that follows, the actual observed units of measurement are used and orthogonal polynomials are employed in the analysis of variance.

ANALYSIS OF RESPONSE SURFACES

Experimental Design

The choice of the points in the factor space can play an important role in determining whether an experimenter is actually observing variables that will provide consistent estimates of the parameters in the model as well as providing maximum information for the number of points used. With linear models a number of important designs have been developed for use in response surface analysis besides the standard factorial and fractional factorial designs. Second order surfaces require a 3^k factorial design which may be replaced by a *composite design* consisting of a 2^k factorial design with extra determinable points added. *Rotatable designs* are intended to provide approximately uniform variance at any given distance from the centre of the design.

Since the design depends entirely upon the model under consideration, and since distributional problems exist with nonlinear models, little progress has been made in developing designs for nonlinear models. Box and Hunter

(1963, 1964) have discussed some methods of designing experiments for nonlinear models. Much work remains to be done in this area.

Estimation of the Parameters

The maximum likelihood estimates of the parameters are those values of the parameters that make the likelihood function a maximum. In the model under consideration, the likelihood function is given by equation 13. The maximum likelihood estimates are determined by setting the first derivatives of $L(\gamma,\boldsymbol{\beta},\boldsymbol{\alpha})$ with respect to the parameters equal to zero and solving the equations so obtained. With the nonlinear model the resulting equations are nonlinear in the parameters, and some iterative procedure such as Newton's method must be used to determine the solutions. Various methods have been devised that are efficient with certain models or with certain data or with both (see Draper and Smith (1966) for some of the more important cases). A three-step linearization procedure that has been found satisfactory for the model in equation 10 is described below.

A linear transformation of the response variable simplifies the likelihood function by eliminating the Jacobian. Let the geometric mean, $\prod_{i=1}^{n} y_i^{1/n}$, for the response observations be denoted by $\dot{y}$. Then, apply the transformation

$$y \rightarrow y^{\gamma} \rightarrow \frac{y^{\gamma} - 1}{\gamma\, \dot{y}^{\gamma-1}}. \tag{15}$$

The Jacobian of the complete transformation is then equal to unity and the new variable is continuous at $\gamma = 0$. This linear transformation of y^{γ} will not affect the maximum likelihood estimate of γ. A similar linear transformation may be applied to the x_j

$$x_j \rightarrow x_j^{\alpha_j} \rightarrow \frac{x_j^{\alpha_j} - 1}{\alpha_j}$$

for continuity at the origin.

The family of transformations then becomes

$$y \rightarrow \frac{y^{\gamma} - 1}{\gamma \dot{y}^{\gamma-1}} = y^{(\gamma)} \quad \gamma \neq 0, \pm \infty \tag{16}$$

$$x \rightarrow \frac{x^{\alpha} - 1}{\alpha} = x^{(\alpha)} \quad \alpha \neq 0, \pm \infty. \tag{17}$$

Denote these transformations, which will be used in this section on parameter estimation, by $y^{(\gamma)}$ and $x^{(\alpha)}$.

In an iterative procedure, initial estimates $\boldsymbol{\alpha}_o$ and γ_o of the power parameters in the model must be supplied. These may be based on some previous

knowledge of the model; however, initial estimates of unity have proven adequate using the estimation procedure here discussed.

The three-step procedure is as follows:

Step 1 — After substituting the initial estimates of the power parameters into the model in equation 10, the maximum likelihood estimates of the β_j coefficient parameters are calculated. These estimates are not the true maximum likelihood estimates of these parameters, since they are conditional on the power parameters being set at the initial estimates. This estimation step is relatively simple since the resulting equations, setting the first derivatives of the likelihood with respect to the coefficients equal to zero, are linear and are easily solved.

Step II — The functional relationship in equation 10 is linearized with respect to the power parameters of the factor space (see Draper and Smith, 1966). This is equivalent to taking terms up to first order in the Taylor expansion of $f(\mathbf{x}_i,\boldsymbol{\beta},\boldsymbol{\alpha})$ about the initial estimates, $\boldsymbol{\alpha}_o$. The resulting equation is

$$y_i^{(\gamma_o)} = f(\mathbf{x}_i,\boldsymbol{\beta},\boldsymbol{\alpha}_o) + \sum_{j=1}^{m}(\alpha_{j1} - \alpha_{jo})f_{\alpha_j}(\mathbf{x}_i,\boldsymbol{\beta},\boldsymbol{\alpha}_o)$$

$$= \beta_o + \sum_{j=1}^{m}\beta_j x_{ij}^{(\alpha_{jo})} + \sum_{j=1}^{m}\sum_{k=j}^{m}\beta_{jk}x_{ij}^{(\alpha_{jo})}x_{ik}^{(\alpha_{ko})}$$

$$+ \sum_{j=1}^{m}(\alpha_{j1} - \alpha_{jo})(\beta_j x_{ij}^{(\alpha_{jo})} + 2\beta_{jj}x_{ij}^{(2\alpha_{jo})} + \sum_{k>j}^{m}\beta_{jk}x_{ij}^{(\alpha_{jo})}x_{ik}^{(\alpha_{ko})})\log_e x_{ij}. \quad (18)$$

Now $$(\beta_j x_{ij}^{(\alpha_{jo})} + 2\beta_{jj}x_{ij}^{(2\alpha_{jo})} + \sum_{k>j}^{m}\beta_{jk}x_{ij}^{(\alpha_{jo})}x_{ik}^{(\alpha_{ko})})\log_e x_j$$

may be considered as new variables and the $(\alpha_{j1}-\alpha_{jo})$ as new parameters for which maximum likelihood estimates may be calculated as in step I, solving only linear equations. The new estimates of the power parameters follow immediately since maximum likelihood estimation is functionally invariant. Throughout step II, the values of $\boldsymbol{\beta}$ obtained in step I are used.

Step III — The model now stands as

$$y_i^{(\gamma_o)} = \beta_o + \sum_{j=1}^{m}\beta_j x_{ij}^{(\alpha_{j1})} + \sum_{j=1}^{m}\sum_{k=j}^{m}\beta_{jk}x_{ij}^{(\alpha_{j1})}x_{ij}^{(\alpha_{k1})} \quad (19)$$

where $\boldsymbol{\beta}$ is obtained from step I and $\boldsymbol{\alpha}$ from step II. Since much of the computation time in this procedure involves calculating cross product matrices, it has been found more efficient to use the same $\boldsymbol{\alpha}$ as in step I at this stage, instead of recalculating $\boldsymbol{\beta}$ for the new $\boldsymbol{\alpha}$ after step II. This usually causes a few more iterations to be required for convergence, but the total number of actual calculations, and therefore the computer time, is reduced.

At this stage, the right hand side of equation 19 can be considered as a new dependent variable and $y^{(\gamma)}$ linearized as in step II. This yields the relationship:

$$\beta_o + \sum_{j=1}^{m} \beta_j x_{ij}^{(\alpha_{jo})} + \sum_{j=1}^{m} \sum_{k=j}^{m} \beta_{jk} x_{ij}^{(\alpha_{jo})} x_{ik}^{(\alpha_{ko})}$$

$$= \frac{y_i^{\gamma_o} - 1}{\gamma_o \dot{y}^{\gamma_o - 1}} + (\gamma_1 - \gamma_o) \left[\frac{y_i^{\gamma_o} \log y_i}{\gamma_o \dot{y}^{\gamma_o - 1}} - \frac{y_i^{\gamma_o} - 1}{\gamma_o^2 \dot{y}^{\gamma_o - 1}} - \frac{(y_i^{\gamma_o} - 1) \log \dot{y}}{\gamma_o \dot{y}^{\gamma_o - 1}} \right]. \quad (20)$$

The estimation of the parameter $(\gamma_1 - \gamma_o)$ yields a new estimate of γ.

Steps I, II, and III are repeated, using the values of α and γ from steps II and III as new initial estimates in step I of each iteration, until some convergence criterion is met, i.e., until the change in each of the parameters between successive iterations is sufficiently small.

In most examples tried, using two- and three-factor models, convergence to three or more digits occurred within about 10 iterations. Exceptions involved models where the relative likelihood graphs of the parameters were very flat, hence pointing to no definite power parameter values.

The method of calculating the maximum likelihood estimates, with appropriate modifications, is also used for estimating the parameters for the maximized relative likelihood. A restraint is placed on the iteration technique so that one of the parameters remains at a fixed value. The estimates so obtained are then substituted in the relative likelihood function.

Adequacy of the Model

The linear polynomial response model (equation 10 with $\gamma = 1 = \alpha_j$) can be considered to be a second order approximation of the Taylor series expansion of some unknown function. For a given number of experimental points the mean sum of squares for deviations from the model (lack of fit) provides a measure of the adequacy of the model as a whole in describing the phenomenon under consideration. In addition, Box (1954) has emphasized that the experimental design should allow the estimation of some factors of higher order than that of the linear model, in order to give an indication of adequacy of the approximation.

If the model with transformations of the variables is actually considered as a new model with a change of units of measurement, both of the above procedures should be taken into consideration and additional terms in the Taylor series added, if necessary, for the next experiment. A large lack of fit mean sum of squares for a nonlinear model could also indicate that the model must be revised; it is possible to gain insight as to possible courses of revision by noting the behaviour of the likelihood function under variation of the existing parameters.

If more than one observation is taken at each point in the factor space, by a true replication of the experiment or by several observations per cell

within the experiment, some measure of experimental error is obtained by fitting the highest degree polynomial through the points (using all of the degrees of freedom). The relative likelihood of the model in equation 10 under consideration versus this model indicates the adequacy of fit of the model in equation 10.

Box and Hunter (1965) have suggested that an indication of desired changes in the model may be discovered by making several runs of an experiment using different points in the factor space at each run. The estimates of the parameters in the model are given by $\hat{\theta}$ which is calculated from the combined observations for all runs. In addition $\hat{\theta}_i$ may be calculated from each run i, and the relative likelihood of $\hat{\theta}$ versus each $\hat{\theta}_i$ used to discover if there exists any significant relation of the run estimates of θ to the location of the observation points. If such a relation is discovered, appropriate modifications will need to be made to the model. The relation of expected to observed responses also provides a useful indication of possible inadequacy of the model. See also Box and Hunter (1962).

Plotting the Surface

As Box and his colleagues have consistently pointed out, with a linear response surface model, canonical analysis of the model and plotting of the contours is extremely important in understanding the surface with which one is working. With nonlinear models, canonical analysis yields little information about the shape of the surface under consideration, but it does provide a simple technique for calculating points to plot on the surface. Also, with linear models, the centre of the surface is usually very important to an understanding of the response surface.

In the nonlinear model, the centre may not always occur near the region of the factor space under consideration. If the eigenvalues of the coefficient matrix in the canonical analysis are of differing sign, as discussed below, there may be a *local* maximum of the fitted surface in the region of interest. The following is a simple procedure for determining the centre of the fitted surface.

Let $\mathrm{B} = (\zeta_{jk}\beta_{jk})$ be the matrix of coefficients in the model in equation 10, where $\zeta_{jk} = 1$ if $j = k$, and $\zeta_{jk} = \frac{1}{2}$ if $j \neq k$; let $\mathrm{C} = (\frac{1}{2}\beta_j)$ be the vector of 0.5 times the coefficients in the model in equation 10, and let $\mathrm{X} = (x_{sj}^{\alpha_j})$ be the vector of the centre point. The vector of the centre coordinates is found by solving the set of simultaneous linear equations

$$\mathrm{BX} = \mathrm{C} \tag{21}$$

and the response centre is given by

$$y_s^{\gamma} = \beta_o + \mathrm{X'C}. \tag{22}$$

The canonical analysis of the surface depends upon the eigenvalues and eigenvectors of the matrix of coefficients B, which will be denoted by $\boldsymbol{\lambda}' = (\lambda_1,$

$\ldots, \lambda_m$) and by $\mathbf{v}_i' = (v_{1i}, \ldots, v_{mi})$, $i = 1, \ldots, m$, respectively. Canonical variables are derived:

$$z_{ik} = \sum_{j=1}^{m} (x_{ij}^{\alpha j} - x_{sj}^{\alpha j}) v_{kj}. \tag{23}$$

The point $\mathbf{x}_i$ in the factor space is transformed to the point $\mathbf{z}_i = (z_{i1}, \ldots z_{im})$ in the canonical space, which has its origin at the centre of the surface and its axes along the principal axes of the conical in the transformed variables, $x_{ij}^{\alpha j}$. The canonical equation of the surface is

$$y_i^{\gamma} - y_s^{\gamma} = \sum_{j=1}^{m} \lambda_j z_{ij}^2. \tag{24}$$

It should be clear that equation 10 is a conical if the units of the coordinates are considered to be $x_{ij}^{\alpha j}$. Equation 24 then shows the conical shape of the surface in terms of these units. When the surface is considered in terms of the original units x_{ij}, it is of course nonlinear and not conical.

If all of the λ_i have the same sign, some optimum (maximum or minimum) will have been reached in terms of the x_{ij} factor space although not necessarily in the sampled region. If the eigenvalues are of differing sign various nonlinear surfaces are possible, perhaps with a local optimum in the region of interest. No easy interpretation is available, as in the linear case; the eigenvalues do not express the significance of the factors in a straightforward manner because they do not take into account the power parameters of the model. The most valuable function of canonical analysis in the nonlinear case is its use in the calculation of points for plotting.

Since an $(m + 1)$-dimensional space is involved in the study of response surfaces, difficulties arise in viewing the entire space when $m > 2$. When $m = 2$, contours of various levels of the response may be plotted on coordinates of the two factors. In higher dimensional spaces, slices must be taken on hyperplanes to obtain a surface suitable for plotting in two dimensions. For example, with $m = 3$, the plane

$$x_1 = k_1 \tag{25}$$

might be considered and contours of y plotted on the x_2,x_3-coordinates along this plane. In terms of the model, substituting the constraints of the plane of the slice into equation 10 will yield an equation with only two factors varying, i.e., a surface in three dimensions. Thus all plotting of surfaces, by means of slices, can be considered in terms of the two-factor response surface. The method of calculation of contours outlined below provides the calculations for 40 points (x_{i1}', x_{i2}') on each response contour $y = y_k$, but the number of points can easily be altered (Lindsey, 1968).

When the constraints of the hyperplane are substituted into equation 10, two eigenvalues λ_1', λ_2' for the matrix of coefficients may be obtained. Let

$$u_{1,i} = [(y_k^\gamma - y_s^\gamma)/\lambda_1']^{\frac{1}{2}} \cos[(i-1)\pi/20] \quad i = 1,\ldots,11 \tag{26}$$

if the eigenvalues have the same sign and

$$u_{1,i} = x_{l1}^{\alpha_1} + [(y_k^\gamma - y_s^\gamma)/\lambda_1']^{\frac{1}{2}} - x_{l1}^{\alpha_1}\cos[(i-1)\pi/20] \quad i = 1,\ldots,11 \tag{27}$$

if they are of opposite sign where x_{l1} is a limit on the size of the x_1 factor. Also,

$$u_{2,i} = [(y_k^\gamma - y_s^\gamma - \lambda_1' u_{1,i}^2)/\lambda_2]^{\frac{1}{2}} \quad i = 1,\ldots,11. \tag{28}$$

Then,

$$\begin{aligned} u_{1,i} &= u_{1,42-i} \\ &= -u_{1,20+i} \qquad i = 1,\ldots,11 \\ &= -u_{1,22-i} \end{aligned} \tag{29}$$

and

$$\begin{aligned} u_{2,i} &= -u_{2,42-i} \\ &= u_{2,20+i} \qquad i = 1,\ldots,11. \\ &= u_{2,22-i} \end{aligned} \tag{30}$$

The points are then given by

$$\begin{aligned} x_{i1}^{\alpha_1} &= v_{11}' u_{1,i} + v_{21}' u_{2,i} + x_{s1}^{\alpha_1} \\ x_{i2}^{\alpha_2} &= v_{12}' u_{1,i} + v_{22}' u_{2,i} + x_{s2}^{\alpha_2}. \end{aligned} \tag{31}$$

Throughout equations 26 to 31, x_{i1} and x_{i2} signify the first and second remaining variable factors after the constraints of the slice are applied to the model in equation 10. If equation 26 is used, only response contours below the maximum (above the minimum) will be calculated, whereas with equation 27 contours both above and below are used.

Inferences re the Parameters

Customarily, the statistician has seemed concerned with only two points in inferences about the parameters of a model: the maximum likelihood (or unbiased minimum variance, etc.) estimate, and the point estimate where the parameter disappears from the model. In the present case this is exemplified by the use of the analysis of variance table, which contains tests of the possibility of various parameters and combinations of parameters being zero. This is an important first step, which will be dealt with; nevertheless to understand the model more completely, the effect of variation of the parameter values on the model will be considered through the use of the likelihood function. This can be accomplished by use of contour plots of relative likelihoods, and by use of maximized relative likelihoods.

In the case of the nonlinear model, the standard F-tests are no longer valid in the analysis of variance table, as explained in the introduction, since probabilities for the ratios of sums of squares cannot be obtained from existing

tables. It is a simple matter to replace the column of F-values in the analysis of variance table by a column of relative likelihoods of the various parameters or combinations of parameters being zero. These provide exact inferences regarding the effects, as the F-statistics do in the linear model. Of course such a likelihood table (e.g., Tables 2A, 4A, 6A) can be set up for the linear model, and this is very useful in familiarizing oneself with the properties of the analysis of variance tables in the nonlinear case.

In order to carry out the inference procedures described above as independently as possible, orthogonal polynomials must be used. In most designs using a nonlinear model, it is only possible to orthogonalize the $x_{ij}^{\alpha_j}$ as opposed to the $x_{ij}^{2\alpha_j}$ effects (i.e., uncorrelated) and not between factors, even though this is desirable. Robson (1959) has reported a simple method of calculating the polynomials when the spacing between levels of a factor, $x_{ij}^{\alpha_j}$, are unequal.

The orthogonal polynomials are calculated as follows:

(i) $x_{ij}^{\alpha_j}$ linear: $f_1(x_{ij}^{\alpha_j}) = (x_{ij}^{\alpha_j} - \overline{x_j^{\alpha_j}})/[\sum_i (x_{ij}^{\alpha_j} - \overline{x_j^{\alpha_j}})^2]^{\frac{1}{2}}$ (32)

(ii) $x_{ij}^{2\alpha_j}$ quadratic: $f_2(x_{ij}^{\alpha_j}) = x_{ij}^{2\alpha_j} - \frac{1}{n}\sum_i x_{ij}^{2\alpha_j} - \frac{(x_{ij}^{\alpha_j} - \overline{x_j^{\alpha_j}})\sum_i x^{2\alpha_j}(x_{ij}^{\alpha_j} - \overline{x_j^{\alpha_j}})}{\sum_i (x_{ij}^{\alpha_j} - \overline{x_j^{\alpha_j}})}$ (33)

(iii) $x_{ij}^{\alpha_j} x_{ik}^{\alpha_k}$ cross product: $f_1(x_{ij}^{\alpha_j})\, f_1(x_{ik}^{\alpha_k})$.

These polynomials become the new variables in the analysis of variance and the relative likelihoods of their coefficients being zero can be calculated. In the linear case, this procedure is equivalent to the method mentioned by Box in Davies (1956, p. 519) for orthogonalizing the quadratic term.

If the maximized relative likelihood of some coefficient parameter being zero or of some power parameter being unity is high, this will indicate that elimination of that parameter from the model is possible without greatly affecting the adequacy of the model, for values of the other parameters near their maximum likelihood estimates. If the parameter is accepted as necessary to the model, the effect of various values of the other parameters may be considered through their various relative likelihoods. These relative likelihoods may point to an interpretable point in the parameter space that will be of use in considering the relation of the model to the nature of the phenomenon under study. The relative likelihoods also provide a measure of the precision of the maximum likelihood estimates. A steeply peaked graph indicates that the parameter is being estimated with high precision, whereas a flat graph demonstrates that the data provide little information about the parameter.

If the experimenter is primarily interested in determining the optimum response conditions, relative likelihoods may be used to give an idea of the precision of the estimates of the factor coordinates of the centre. The procedure outlined below for doing this may also be used in the linear case for which, in addition, fiducial and confidence intervals can be calculated (see Box and Hunter, 1954).

If equation 10 is differentiated with respect to the various factors, a system of linear equations in the transformed centre coordinates is obtained (equation 21):

$$\frac{\partial y^{\gamma}}{\partial x_{ij}} = \alpha_j \beta_j x_{sj}^{\alpha_j - 1} + 2\alpha_j \beta_{jj} x_{sj}^{2\alpha_j - 1} + \alpha_j \sum_{k \neq j}^{m} \beta_{jk} x_{sj}^{\alpha_j - 1} x_{sk}^{\alpha_k}$$

$$= 0$$

$$= \beta_j + 2\beta_{jj} x_{sj}^{\alpha_j} + \sum_{k \neq j}^{m} \beta_{jk} x_{sk}^{\alpha_j}. \tag{34}$$

These constraint equations can be used with Lagrange multipliers with equation 10 or they can be substituted directly into equation 10 eliminating the β_j coefficients, yielding

$$y_i = \beta_o + \sum_{j=1}^{m} \sum_{k=j}^{m} \beta_{jk} (x_{ij}^{\alpha_j} x_{ik}^{\alpha_k} - x_{ij}^{\alpha_j} x_{sk}^{\alpha_k} - x_{sj}^{\alpha_j} x_{ik}^{\alpha_k}). \tag{35}$$

Since $\mathbf{x}_s = (x_{s1}, \ldots, x_{sm})$ is the maximum likelihood estimate of the centre of the surface, the relative likelihoods of various possible centre points may be calculated. Maximized relative likelihood graphs can be plotted for each of the coordinates of the centre. As seen in the examples, if a maximum or a minimum does not occur, the precision of the estimate of the centre can be very low.

The inference procedures outlined above are also valid for the analogous linear model, although corresponding probability distributions of the parameters can be found. As well, the procedures with suitable modifications are valid with other assumptions than those of equation 3. Anscombe (1961) gives an excellent discussion of likelihood inference procedures in a nonnormal situation as do Feigl and Zelen (1965). In examples of the designed two-factor experiment and the designed three-factor experiment below, a binomial assumption with $E(y_i/100) = p_i = f(\mathbf{x}_i, \boldsymbol{\theta})$ might be more reasonable (where P_i is the probability of an egg hatching under conditions $\mathbf{x}_i$). The methods of this paper are easily adapted to other nonlinear situations.

A computer program has been developed using FORTRAN on an IBM 1130 computer, which will handle the complete analysis as described above, for two- and three-factor models (Lindsey, 1968). This programme was used for the computations in the examples that follow.

EXAMPLES

Designed Two-Factor Experiment

Alderdice and Forrester (1968) performed an experiment to determine the effects of variation in salinity (x_1‰ S) and temperature (x_2 C) of sea water on the percent total hatch of eggs of the English sole (*Parophrys vetulus*). A

17-point design was used, which gave correlated estimates only of the β_{jj} parameters in the linear model. Four observations were made at each experimental point in the factor space (Table 1). The fitted linear model is

$$y = -186.6 + 7.1\,x_1 + 43.4\,x_2 - 0.2\,x_1^2 - 2.7\,x_2^2 + 0.2\,x_1x_2. \qquad (36)$$

TABLE 1. Percentage hatch of English sole eggs incubated under 17 different salinity–temperature conditions.

Variables fitted		Observation sets				Response		
Salinity x_1 (‰)	Temperature x_2 (C)	1	2	3	4	Mean	Variance	SD[a]
15.00	4.00	35.60	28.20	24.10	29.48	29.34	67.85	8.23
15.00	8.00	91.62	93.46	82.60	91.33	89.75	70.98	8.42
15.00	12.00	72.07	57.04	65.33	76.81	67.81	221.24	14.87
25.00	4.00	28.47	22.79	18.35	23.54	23.28	51.55	7.18
25.00	8.00	95.33	88.29	94.85	93.49	92.98	31.27	5.59
25.00	12.00	76.52	72.25	83.38	78.17	77.58	63.49	7.96
35.00	4.00	0.30	0.62	1.51	0.56	0.74	0.83	0.91
35.00	8.00	85.55	89.88	84.87	61.88	80.54	479.28	21.89
35.00	12.00	75.30	73.80	66.60	83.88	74.76	142.10	11.92
10.00	10.00	44.65	46.50	43.04	43.16	44.33	7.84	2.80
10.00	6.00	36.75	35.40	40.96	36.99	37.52	17.20	4.14
40.00	10.00	86.16	70.50	68.94	52.81	69.60	557.37	23.60
40.00	6.00	41.85	45.73	47.21	25.37	40.04	302.41	17.39
20.00	10.00	89.93	86.16	87.56	87.01	87.66	7.82	2.79
20.00	6.00	91.14	92.56	88.08	86.67	89.61	21.99	4.69
30.00	10.00	91.23	89.17	90.21	91.12	90.43	2.75	1.65
30.00	6.00	84.68	85.39	85.83	85.21	85.28	0.66	0.81
Within observation sets								
Means		66.30	63.39	63.14	61.58			
Variances		13272.19	13229.60	13260.24	14126.75			
SD		115.20	115.02	115.15	118.85			

[a]SD = standard deviation.

The power parameters in the nonlinear model were calculated to be

$$\hat{\gamma} = 0.8465,\ \hat{\alpha}_1 = 0.1858, \text{ and } \hat{\alpha}_2 = -0.2353$$

so that the fitted nonlinear model is

$$y^{0.85} = -2448.5 + 1527.7\,x_1^{0.19} - 3735.6\,x_2^{-0.24} - 366.5\,x_1^{0.38} - 2540.2\,x_2^{-0.47} - 362.7\,x_1^{0.19}x_2^{-0.24}. \qquad (37)$$

The response centre in the linear model is

$$\mathbf{x}_s = (25.9‰\ \text{S}, 9.1\ \text{C}) \text{ with } y_s = 103.5\% \text{ hatch}$$

whereas in the nonlinear model

$$\mathbf{x}_s = (22.5‰\ \text{S}, 8.3\ \text{C}) \text{ and } y_s = 100.2\% \text{ hatch}.$$

The canonical equation in the linear model is

$$y - 103.5 = -2.68\, z_1^2 - 0.17\, z_2^2.$$

Contour plots of the surfaces are given in Fig. 1A, B.

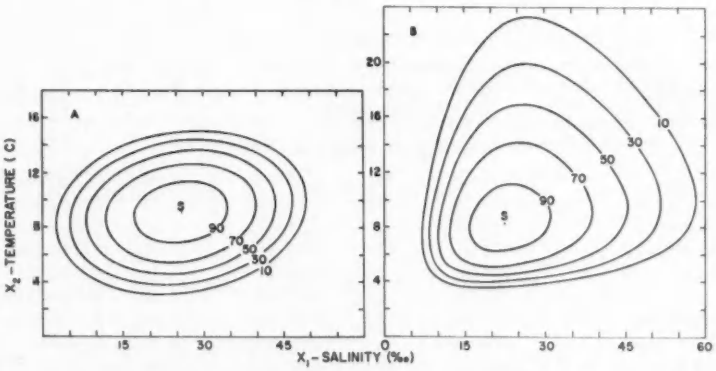


FIG. 1. Response surface contours for the English sole data of Table 1. A, According to the model in equation 36. B, According to the model in equation 37.

The relative likelihood of the linear model as opposed to the nonlinear is 4.0×10^{-11}. The maximized relative likelihood (Fig. 2C) of $\gamma = 1$ is about $R(\gamma = 1) = 0.25$ so that γ can possibly be eliminated. As seen from the graphs (Fig. 2A, B), the corresponding maximized relative likelihoods of the other

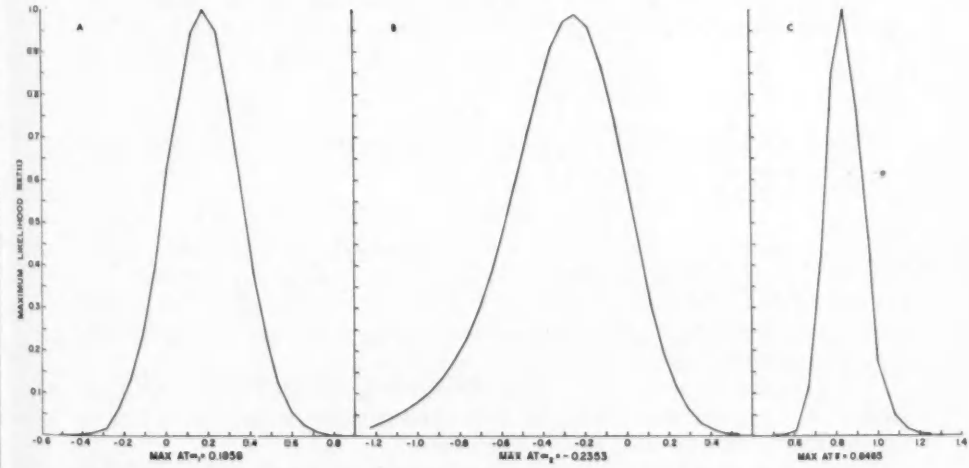


FIG. 2. Graphs of the maximized relative likelihood ratios of the power parameters for the English sole data of Table 1. A, Parameter α_1. B, Parameter α_2. C, Parameter γ. (Model, equation 37.)

power parameters equaling unity are very low. Analyses of variance for the two models are given in Table 2. The F-statistics are the standard tests of an analysis of variance, whereas the relative likelihoods are tests for the given coefficient parameter(s) being zero, given the model and the data. Orthogonal polynomials were used in calculating both tables. It appears unlikely that

TABLE 2. Analysis of variance for the data of Table 1.

Source	SS[a]	df	MSS[a]	F[b]	MLR[a]
A. According to model in equation 36					
Treatments	52039.33	16	3252.45	81.04	0.511838E–48[c]
Linear	17429.59	2	8714.79	217.15	0.322044E–18
x_1 linear	25.18	1	25.18	0.62	0.884696E–00
x_2 linear	17404.41	1	17404.41	433.68	0.333548E–18
Quadratic	27859.88	2	13929.94	347.10	0.179322E–23
x_1 quadratic	13471.47	1	13471.47	335.68	0.132071E–15
x_2 quadratic	19393.70	1	19393.70	483.25	0.231725E–19
x_1x_2 cross product	1820.60	1	1820.60	45.36	0.376612E–03
Lack of fit	4929.25	11	448.11	11.16	0.782162E–18
Residual	2046.69	51	40.13		
Total	54086.03	67			
B. According to model in equation 37					
Treatments	53488.18	16	3343.01	89.40	0.194009E–49
Linear	26495.92	2	13247.96	354.28	0.123167E–31
x_1 linear	332.24	1	332.24	8.88	0.438899E–01
x_2 linear	26208.61	1	26208.61	700.88	0.170940E–31
Quadratic	23722.45	2	11861.22	317.20	0.335422E–30
x_1 quadratic	13965.62	1	13965.62	373.47	0.124175E–23
x_2 quadratic	14515.55	1	14515.55	388.18	0.431505E–24
x_1x_2 cross product	2119.17	1	2119.17	56.67	0.849201E–07
Lack of fit	1542.97	8	192.87	5.15	0.176366E–11
Residual	1907.06	51	37.39		
Total	55395.25	67			

[a]SS = sum of squares; MSS = mean sum of squares; MLR = maximum likelihood ratio.
[b]The F-values are approximate for the model in equation 37.
[c]i.e., 0.511838×10^{-48}, etc.

any of the coefficient parameters should be eliminated in the nonlinear model. The maximized relative likelihood graphs of the coefficient parameters are not reproduced here. Nevertheless they would be considered at this point, as well as the graphs for the power parameters, to determine what estimates of the parameters are compatible with the data.

The maximized relative likelihood graphs (Fig. 3A, B, for the nonlinear model) of the centre coordinates for both models indicate that the centre is known with great precision, given the model.

If the maximum likelihood estimates of the parameters are used, it appears that the nonlinear model describes the observed responses fairly well. This

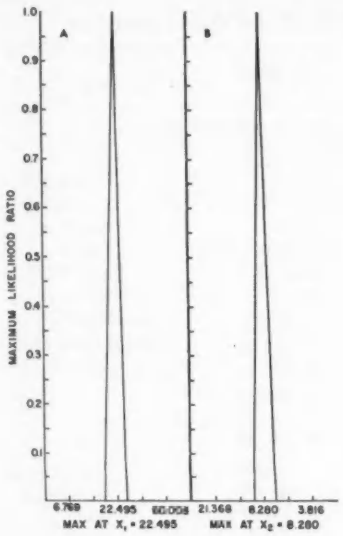


FIG. 3. Graphs of the maximized relative likelihood ratios of the centre coordinates of the nonlinear response surface model in equation 37 for the English sole data of Table 1 for the (A) x_1-coordinate and (B) x_2-coordinate.

can only be considered an empirical model and there is no obvious indication of refinement.

TWO-FACTOR EXPERIMENT WITH CORRELATED ESTIMATES

Fry and Hart (1948) performed experiments on the effects of acclimation (x_1) and experimental (x_2) temperature experience on the swimming speed of the goldfish (*Carassius auratus*). Their results are amenable to response surface analysis, although a designed experiment was not used. The data are reproduced in Table 3. The fitted linear model is

$$y = 21.84 + 0.97\,x_1 + 4.49\,x_2 - 0.18\,x_1^2 - 0.21\,x_2^2 + 0.28\,x_1x_2. \quad (38)$$

Maximum likelihood estimates of the power parameters were calculated to be

$$\hat{\gamma} = 0.1080,\ \hat{\alpha}_1 = 1.6344, \text{ and } \hat{\alpha}_2 = 1.2939$$

so that the fitted nonlinear model is

$$y^{0.11} = 1.48 - 6 \times 10^{-5}\,x_1^{1.63} + 5 \times 10^{-3}x_2^{1.29} - 5 \times 10^{-6}x_1^{3.26} - 1 \times 10^{-5}x_2^{2.58} + 3 \times 10^{-5}x_1^{1.63}x_2^{1.29}. \quad (39)$$

The response centre in the linear model is

$$\mathbf{x}_s = (22.8\text{ C}, 26.6\text{ C}) \text{ with } y_s = 92.6 \text{ ft/min}$$

and it is

$$\mathbf{x}_s = (26.2\text{ C}, 27.5\text{ C}) \text{ with } y_s = 99.9 \text{ ft/min}$$

in the nonlinear model. The canonical equation in the linear model is

$$y - 92.6 = -0.335z_1^2 - 0.054z_2^2.$$

TABLE 3. Cruising speed of goldfish for 22 different acclimation-temperature (x_1) and test-temperature (x_2) conditions. (Data from Fry and Hart, 1948.)

Variables fitted		Cruising speed
x_1 (C)	x_2 (C)	(ft/min)
5.00	5.00	44.00
5.00	10.00	58.00
5.00	15.00	68.00
5.00	20.00	68.00
5.00	25.00	41.00
10.00	10.00	57.00
15.00	5.00	44.00
15.00	15.00	71.00
15.00	20.00	79.00
15.00	25.00	77.00
20.00	20.00	100.00
25.00	15.00	55.00
25.00	20.00	79.00
25.00	25.00	96.00
25.00	30.00	100.00
25.00	35.00	68.00
30.00	30.00	98.00
35.00	20.00	30.00
35.00	25.00	58.00
35.00	30.00	70.00
35.00	35.00	76.00
35.00	38.00	84.00
Mean		69.13
Variance		8174.59
Standard deviation		90.41

Contour plots of the two surfaces are given in Fig. 4A, B. The relative likelihood of the linear model as opposed to the nonlinear model is 9.0×10^{-6}. The maximized relative likelihoods (Fig. 5A–C) of the powers being unity are $R(\alpha_1 = 1) = 0.0004$, $R(\alpha_2 = 1) = 0.05$, and $R(\gamma = 1) = 0.008$; it appears that none of the power parameters could be eliminated from the model. The maximized relative likelihood of $\gamma = 0.0$ or a logarithmic transformation on y is high ($R(\gamma = 0.0) \sim 1.0$), whereas $R(\alpha_1 = 2) = 0.08$, $R(\alpha_2 = 2) = 0.0001$, $R(\alpha_1 = 1.6, \alpha_2 = 1.3, \gamma = 0.0$, i.e., log $y) \sim 1.0$. Thus, a simplified model might be

$$\log y = \beta_o + \beta_1 x_1^{1.6} + \beta_2 x_2^{1.3} + \beta_{11} x_1^{3.2} + \beta_{22} x_2^{2.6} + \beta_{12} x_1^{1.6} x_2^{1.3}.$$

TABLE 4. Analysis of variance for the data of Table 3.

Source	SS[a]	df	MSS[a]	F[b]	MLR[a]
A. According to model in equation 38					
Regression	7003.62	5	1400.32	19.14	0.521043E-09[c]
Linear	2918.64	2	1459.32	19.94	0.106047E-05
x_1 linear	163.50	1	163.50	2.23	0.237462E-00
x_2 linear	2246.67	1	2246.67	30.69	0.763842E-05
Quadratic	4790.80	2	2395.40	32.73	0.167830E-07
x_1 quadratic	4310.92	1	4310.92	58.90	0.422439E-07
x_2 quadratic	2202.10	1	2202.10	30.08	0.882486E-05
x_1x_2 cross product	2574.30	1	2574.30	37.63	0.166504E-05
Lack of fit	1170.96	16	73.18		
Total	8174.59	21			
B. According to model in equation 39					
Regression	8514.18	5	1702.84	54.36	0.179196E-14
Linear	1147.44	2	573.72	18.31	0.398172E-06
x_1 linear	805.85	1	805.85	25.72	0.609994E-05
x_3 linear	1041.85	1	1041.85	33.26	0.863189E-06
Quadratic	6005.69	2	3002.84	95.86	0.676812E-13
x_1 quadratic	5141.46	1	5141.46	164.13	0.332653E-12
x_3 quadratic	3280.43	1	3280.43	104.72	0.297737E-10
x_1x_3 cross product	4041.49	1	4041.49	129.02	0.378007E-11
Lack of fit	407.21	13	31.32		
Total	8921.42	21			

[a,b,c]As in Table 2.

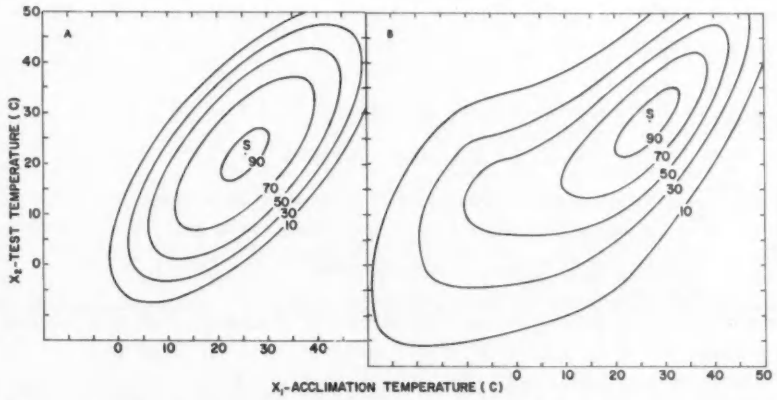


FIG. 4. Response surface contours for the goldfish data of Table 3. A, According to the model in equation 38. B, According to the model in equation 39.

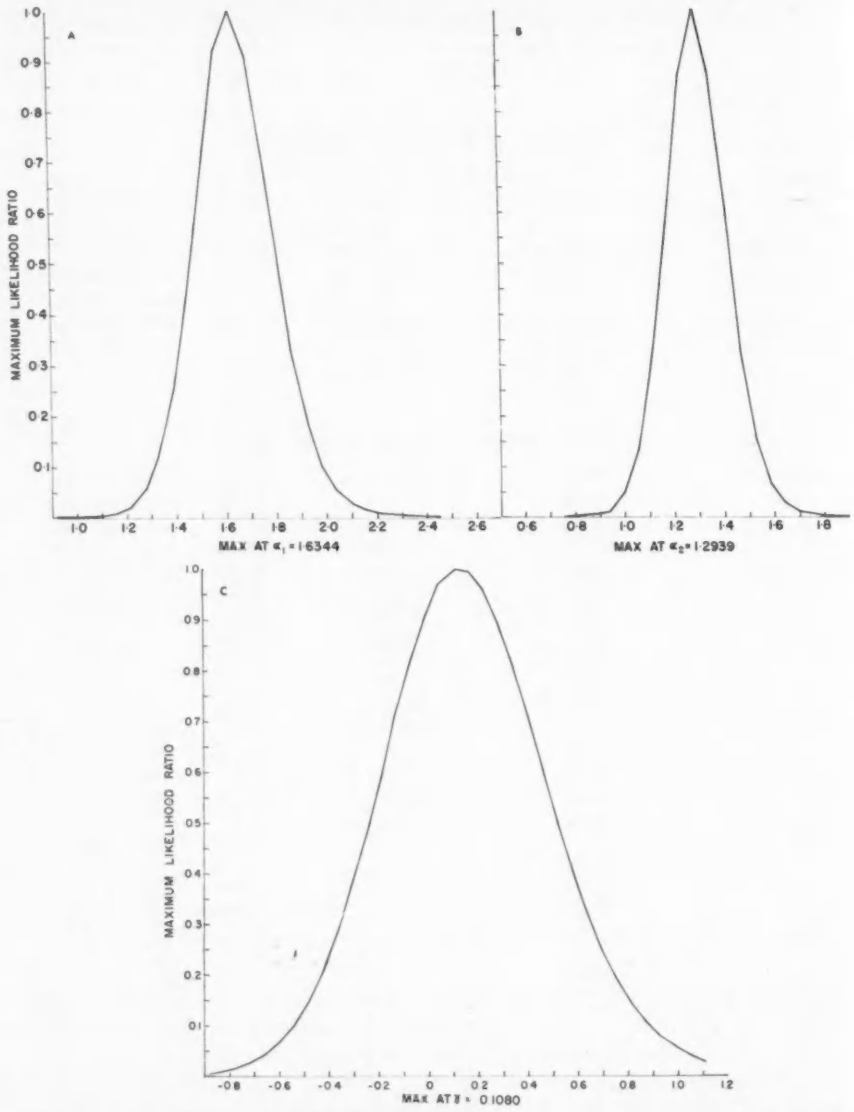


FIG. 5. Graphs of the maximized relative likelihood ratios of the power parameters for the goldfish data of Table 3. A, Parameter α_1. B, Parameter α_2. C, Parameter γ. (Model, equation 39.)

The maximized relative likelihood graphs (Fig. 6A, B) of the centre coordinates show that the estimate of the centre of the surface is not known so precisely as in the first example.

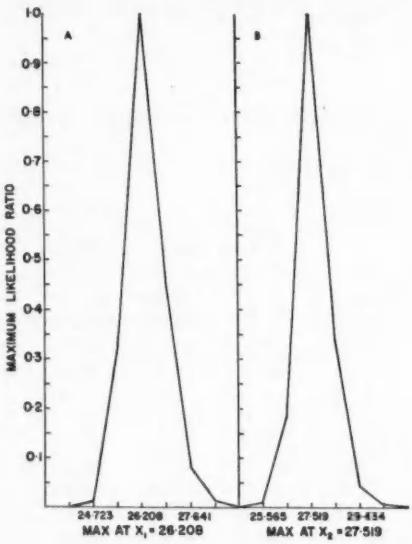


FIG. 6. Graphs of the maximized relative likelihood ratios of the centre coordinates of the nonlinear response surface model in equation 39 for the goldfish data of Table 3 for the (A) x_1-coordinate and (B) x_2-coordinate.

With further analysis, these data might provide the basis of a functional model that could be refined with further experimentation.

DESIGNED THREE-FACTOR EXPERIMENT

Alderdice and Forrester (see the preliminary results in Forrester and Alderdice (1966)) performed experiments to determine the effects of variation in salinity (x_1‰ S), temperature (x_2 C), and oxygen content (x_3 ppm) of sea water on the percent total hatch of eggs of the Pacific cod (*Gadus macrocephalus*). A 15-point design was extended to 19 points to cover the region of the maximum better (Table 5). This caused correlation among many of the coefficient parameters in the fitted linear model, which is

$$y = 55.0 - 4.0x_1 + 33.8x_2 - 6.0x_3 - 0.1x_1^2 - 2.6x_2^2 - 0.2x_3^2 + 0.1x_1x_2 + 0.8x_1x_3 - 0.8x_2x_3. \quad (40)$$

Maximum likelihood estimation by the procedure described in the section on estimation of the parameters discloses a local maximum at

$$\gamma = 0.7661, \alpha_1 = -0.6832, \alpha_2 = -0.5633, \alpha_3 = 2.1939$$

but the maximum likelihood estimates are

$$\hat{\gamma} = 0.5444, \hat{\alpha}_1 = 3.7197, \hat{\alpha}_2 = -0.9455, \text{ and } \hat{\alpha}_3 = -2.1640$$

so that the fitted nonlinear model is

$$y^{0.54} = 46.79 + 2 \times 10^{-5}x_1^{3.72} + 95.35x_2^{-0.95} + 85.31x_3^{-2.16} + 7 \times 10^{-11}x_1^{7.44}$$

$$- 160.60x_2^{-1.89} - 3.08x_3^{-4.33} - 3 \times 10^{-5}x_1^{3.72}x_2^{-0.95} - 7 \times 10^{-4}x_1^{3.72}x_3^{-2.16}$$
$$- 230.00x_2^{-0.95}x_3^{-2.16}. \qquad (41)$$

TABLE 5. Percentage hatch of Pacific cod eggs incubated under 19 salinity (x_1), temperature (x_2), and dissolved oxygen (x_3) conditions.

Variables fitted			Observation sets				Response		
Salinity x_1 (‰)	Temperature x_2 (C)	Dissolved oxygen x_3 (ppm)	1	2	3	4	Mean	Variance	SD[a]
14.00	2.70	3.60	79.16	73.42	68.02	56.92	69.38	269.07	16.40
14.00	2.70	8.60	71.07	83.75	82.50	65.92	75.81	228.04	15.10
14.00	9.30	3.60	66.19	71.10	67.13	76.72	70.29	68.85	8.29
14.00	9.30	8.60	59.29	72.08	32.57	30.28	48.56	1257.94	35.46
26.00	2.70	3.60	2.21	1.64	0.78	1.26	1.47	1.09	1.04
26.00	2.70	8.60	49.00	58.91	52.85	52.25	53.25	51.23	7.15
26.00	9.30	3.60	7.23	6.82	13.01	15.20	10.56	52.46	7.24
26.00	9.30	8.60	25.28	49.56	37.38	26.72	34.73	380.46	19.50
20.00	6.00	6.10	85.31	75.71	85.92	84.44	82.84	69.07	8.31
12.71	6.00	6.10	82.83	81.89	68.18	84.08	79.24	165.43	12.86
27.29	6.00	6.10	19.93	28.87	65.77	52.07	41.66	1325.48	36.40
20.00	2.00	6.10	8.63	4.23	6.07	2.91	5.46	18.44	4.29
20.00	10.00	6.10	33.53	39.63	52.57	41.81	41.88	189.23	13.75
20.00	6.00	3.08	63.84	78.96	65.09	81.27	72.29	248.37	15.75
20.00	6.00	9.14	89.02	91.69	91.48	85.46	89.41	25.31	5.03
20.00	6.00	6.10	56.37	80.28	55.13	88.15	69.98	842.26	29.02
19.28	5.43	12.40	55.88	81.79	84.01	70.62	73.08	497.26	22.29
16.77	5.75	9.46	91.46	86.40	81.62	89.33	87.20	54.53	7.38
14.25	6.05	6.53	85.43	83.90	74.25	82.60	81.55	74.92	8.65
Within observation sets									
Means			54.30	60.56	57.07	57.26			
Variances			15993.35	16091.80	13512.64	15899.25			
SD			126.46	126.85	116.24	126.09			

[a]SD = standard deviation.

The response centre in the linear model is

$$\mathbf{x}_s = (18.1‰ \text{ S}, 5.5 \text{ C}, 9.7 \text{ ppm}) \text{ with } y = 81.9\% \text{ hatch}$$

whereas in the nonlinear model

$$\mathbf{x}_s = (15.6‰ \text{ S}, 3.7 \text{ C}, 9.0 \text{ ppm}) \text{ with } y = 102.4\% \text{ hatch.}$$

The canonical equation in the linear model is

$$y - 81.9 = 0.26\, z_1^2 - 2.64 z_2^2 - 0.48\, z_3^2.$$

Contour plots of slices of the surface, on planes through the response centres, are given in Fig. 7A, B. The relative likelihood of the linear model as opposed to the nonlinear is 5.7×10^{-12}.

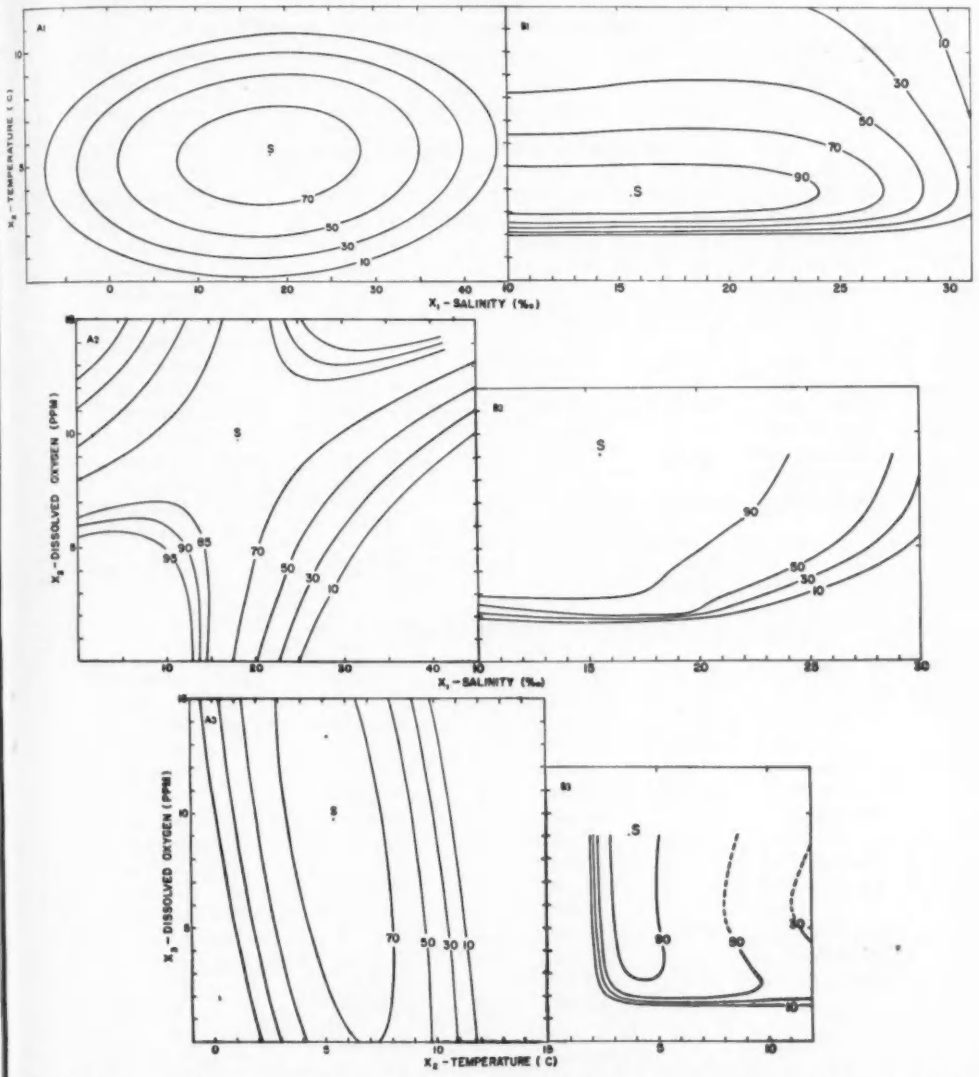


FIG. 7. Response surface contours for the Pacific cod data of Table 5. According to the model in equation 40: (A1) in the x_1,x_2-plane ($x_3 = 9.73$); (A2) in the x_1,x_3-plane ($x_2 = 5.45$); (A3) in the x_2,x_3-plane ($x_1 = 18.13$). According to the model in equation 41: (B1) in the x_1,x_2-plane ($x_3 = 9.02$); (B2) in the x_1,x_3-plane ($x_2 = 3.72$); (B3) in the x_2,x_3-plane ($x_1 = 15.59$).

The maximized relative likelihoods (Fig. 8A–D) of the powers individually equaling unity are all low. The maximized relative likelihoods for the specified values of the power parameters are $R(\gamma = 0.5) \sim 1.0$, $R(\alpha_1 =$

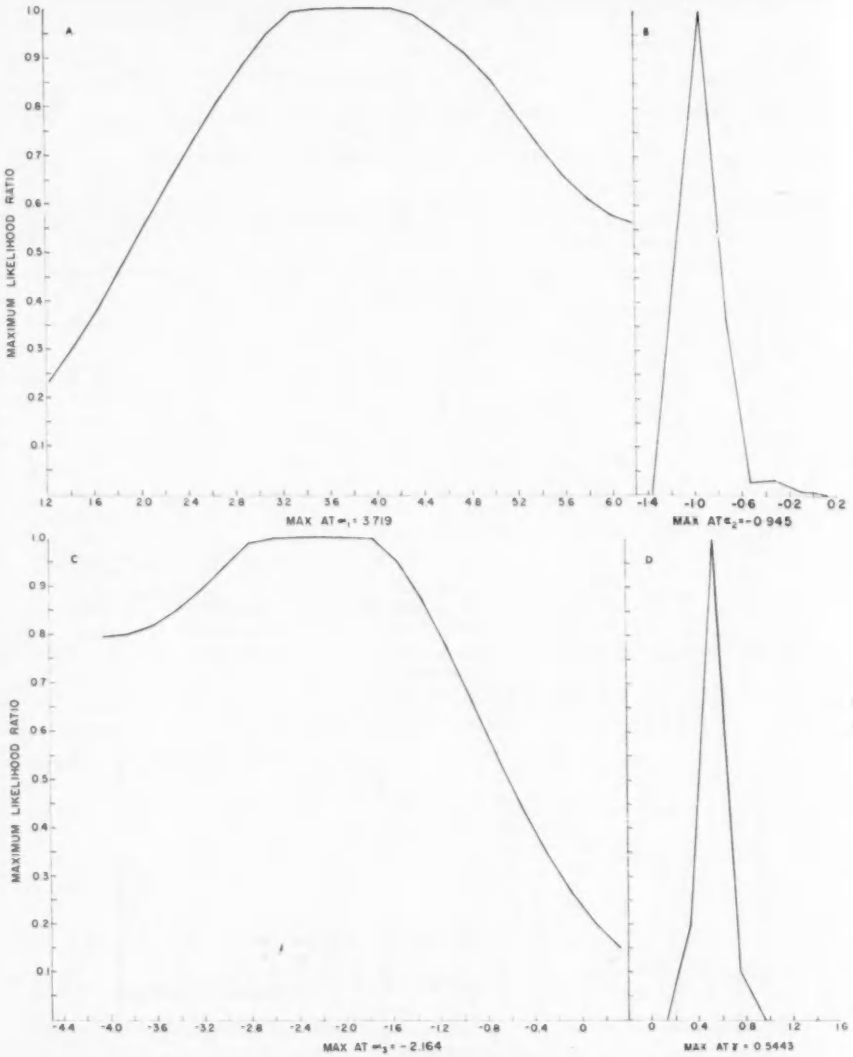


FIG. 8. Graphs of the maximized relative likelihood ratios of the power parameters for the Pacific cod data of Table 5. A, Parameter α_1. B, Parameter α_2. C, Parameter α_3. D, Parameter γ. (Model, equation 41.)

4.0) = 0.99, R(α_2 = −1.0) = 0.89, and R(α_3 = −2.0) = 0.99. Only β_{11}, β_{33}, and β_{12} have high relative likelihoods of being zero (Table 6B). This yields a simplified model:

$$y^{0.5} = \beta_o + \beta_1 x_1^4 + \beta_2 x_2^{-1} + \beta_3 x_3^{-2} + \beta_{22} x_2^{-2} + \beta_{13} x_1^4 x_3^{-2} + \beta_{23} x_2^{-1} x_3^{-2}.$$

TABLE 6. Analysis of variance for the data of Table 5.

Source	SS[a]	df	MSS[a]	F[b]	MLR[a]
A. According to model in equation 40					
Treatments	56051.74	18	3113.98	30.50	0.122800E-38[c]
Linear	13430.37	3	4476.79	43.84	0.140385E-11
x_1 linear	12060.74	1	12060.74	118.13	0.107891E-10
x_2 linear	42.79	1	42.79	0.41	0.880706E-00
x_3 linear	1061.25	1	1061.25	10.39	0.482695E-01
Quadratic	18363.33	3	6121.11	59.95	0.200263E-14
x_1 quadratic	318.97	1	318.97	3.12	0.391926E-00
x_2 quadratic	17220.87	1	17220.87	168.67	0.828613E-14
x_3 quadratic	123.66	1	123.66	1.21	0.693587E-00
Interaction	5855.63	3	1951.87	7.57	0.596741E-06
x_1x_2 cross product	145.26	1	145.26	1.42	0.650869E-00
x_1x_3 cross product	4364.07	1	4364.07	42.74	0.142038E-04
x_2x_3 cross product	1360.77	1	1360.77	13.32	0.213992E-01
Lack of fit	6962.17	9	773.57	7.57	0.103605E-12
Residual	5819.53	57	102.09		
Total	61871.28	75			
B. According to model in equation 41					
Treatments	68678.49	18	3815.47	43.85	0.298300E-44
Linear	30037.34	3	10012.44	115.09	0.404750E-29
x_1 linear	18524.64	1	18524.64	212.94	0.872163E-23
x_2 linear	6277.40	1	6277.40	72.15	0.200656E-11
x_3 linear	2846.48	1	2846.48	32.72	0.464620E-06
Quadratic	22455.15	3	7485.05	86.04	0.312815E-25
x_1 quadratic	300.23	1	300.23	3.45	0.160410E-00
x_2 quadratic	18724.75	1	18724.75	215.24	0.641127E-23
x_3 quadratic	0.01	1	0.01	0.00	0.999937E-00
Interaction	11990.90	3	3996.96	2.59	0.107824E-17
x_1x_2 cross product	398.94	1	398.94	4.58	0.895520E-01
x_1x_3 cross product	8546.80	1	8546.80	98.24	0.331990E-14
x_2x_3 cross product	2322.92	1	2322.92	26.70	0.461289E-05
Lack of fit	1126.67	5	225.33	2.59	0.417875E-03
Residual	4958.66	57	86.99		
Total	73637.16	75			

[a,b,c]As in Table 2.

The location of the centre of the response surface is not known with high precision (Fig. 9A–C) since there is no unique maximum. With further analysis it may be possible to gain some indication of a functional model, the simplified model given above certainly being empirical.

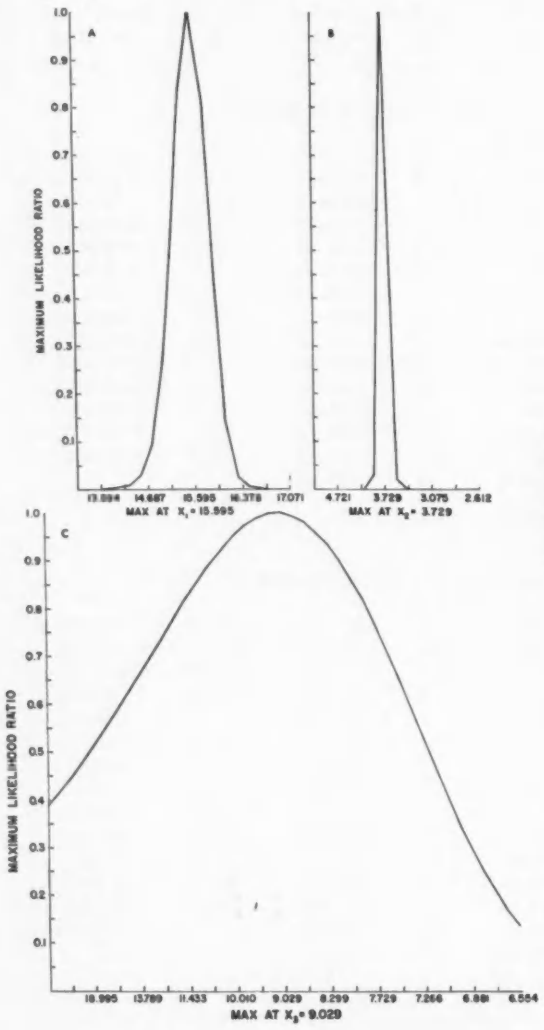


FIG. 9. Graphs of the maximized relative likelihood ratios of the centre coordinates of the non-linear response surface model in equation 41 for the Pacific cod data of Table 5 for the (A) x_1-coordinate, (B) x_2-coordinate, and (C) x_3-coordinate.

ACKNOWLEDGMENTS

The authors express their appreciation for the many helpful comments and suggestions provided by Dean D. A. Sprott, University of Waterloo, Professor D. R. Cox, Imperial College, and Visiting Professor J. G. Kalbfleisch, University of Essex. The staff of the computing centre at the Fisheries Research Board of Canada Biological Station, Nainaimo, B.C., provided invaluable aid in making the computations for the examples.

REFERENCES

ALDERDICE, D. F., AND C. R. FORRESTER. 1968. Some effects of salinity and temperature on early development and survival of the English sole (*Parophrys vetulus*). J. Fish. Res. Bd. Canada 25: 495–521.

ANSCOMBE, F. J. 1961. Estimating a mixed-exponential response law. J. Amer. Statist. Ass. 56: 493–502.

BARNARD, G. A., G. J. JENKENS, AND C. B. WINSTEN. 1962. Likelihood inference and time series. J. Roy. Statist. Soc. Ser. A 125: 321–372.

BEALE, E. M. L. 1960. Confidence regions in non-linear estimation. J. Roy. Statist. Soc. Ser. B 22: 41–88.

BOX, G. E. P. 1954. The exploration and exploitation of response surfaces: some general considerations and examples. Biometrics 10: 16–60.

BOX, G. E. P., AND D. R. COX. 1964. An analysis of transformations. J. Roy. Statist. Soc. Ser. B 26: 211–252.

BOX, G. E. P., AND J. S. HUNTER. 1954. A confidence region for the solution of a set of simultaneous equations with an application to experimental design. Biometrika 41: 190–199.

BOX, G. E. P., AND W. G. HUNTER. 1962. A useful method for model-building. Technometrics 4: 301–318.

1963. Sequential design of experiments for non-linear models. Dep. Statist. Univ. Wis. Tech. Rep. 21.

1964. Non-sequential designs for the estimation of parameters in non-linear models. Dep. Statist. Univ. Wis. Tech. Rep. 28.

1965. The experimental study of physical mechanisms. Technometrics 7: 23–42.

BOX, G. E. P., AND P. W. TIDWELL. 1962. Transformation of the independent variables. Technometrics 4: 531–550.

BOX, G. E. P., AND K. WILSON. 1951. On the experimental attainment of optimum conditions. J. Roy. Statist. Soc. Ser. B 13: 1–45.

DAVIES, O. L. [ed.] 1956. Design and analysis of industrial experiments. Oliver and Boyd, London, England. xiii + 636 p.

DOLBY, J. L. 1963. A quick method for choosing a transformation. Technometrics 5: 317–325.

DRAPER, N. R., AND W. G. HUNTER. 1969. Transformations: some examples revisited. Technometrics 11: 23–40.

DRAPER, N. R., AND H. SMITH. 1969. Applied regression analysis. John Wiley and Sons, New York, N.Y. ix + 407 p.

FEIGL, P., AND M. ZELEN. 1965. Estimation of exponential survival probabilities with concomitant observations. Biometrics 21: 826–838.

FISHER, R. A. 1956. Statistical methods and scientific inference. Oliver and Boyd, London, England.

FORRESTER, C. R., AND D. F. ALDERDICE. 1966. Effects of salinity and temperature on embryonic development of the Pacific cod (*Gadus macrocephalus*). J. Fish. Res. Bd. Canada 23: 319–340.

FRY, F. E. J., AND J. S. HART. 1948. Cruising speed of goldfish in relation to water temperature. J. Fish. Res. Bd. Canada 7: 169–175.

LINDSEY, J. K. 1968. Program for the analysis of non-linear response surfaces. Fish. Res. Board Can. Tech. Rep. 87: 94 p.

ROBSON, D. S. 1959. A simple method for constructing orthogonal polynomials when the independent variable is unequally spaced. Biometrics 15: 187–191.

SPROTT, D. A. MS, 1968. Significance intervals and fiducial tests of significance. Dep. Math. Univ. Waterloo, Waterloo, Ont.

SPROTT, D. A., AND J. D. KALBFLEISCH. 1969. Examples of likelihoods and comparisons with point estimates and large sample approximations. J. Amer. Statist. Ass. 64: 468–484.

Development of Salinity Preference in Pre-Smolt Coho Salmon, *Oncorhynchus kisutch*

ROBERT G. OTTO[1] AND JOHN E. MCINERNEY

Department of Biology
University of Victoria, Victoria, B.C.

OTTO, R. G., AND J. E. MCINERNEY. 1970. Development of salinity preference in pre-smolt coho salmon, *Oncorhynchus kisutch*. J. Fish. Res. Bd. Canada 27: 793–800.

When tested in a horizontal salinity gradient, freshwater-adapted coho salmon exhibited a bimodal preference response throughout most of the pre-smolt period. Preference modes were located at fresh water and at a salinity intermediate between fresh and sea water. The concentration of the higher preferred salinity changed both with time and instantaneously with the dimensions of the test gradient. Fish tested in a gradient that originated at 4‰ exhibited a single preferred salinity at 7‰ in June, which increased gradually to 13‰ by February. The same fish tested in a gradient originating at fresh water showed a corresponding preference for an intermediate salinity in addition to their preference for fresh water. However, this second preferendum was at a somewhat lower salinity than that observed in tests in which fresh water was excluded from the gradient.

With the approach of the smolt transformation, preference for fresh water was greatly reduced and the response distribution became unimodal. At no time during the period prior to the smolt transformation did the preferred salinity exceed 14‰.

Received August 29, 1969

INTRODUCTION

THIS STUDY describes the response of pre-smolt coho salmon (*Oncorhynchus kisutch*) to a salinity gradient. The ability of fish to perceive minor changes in environmental salinity was first demonstrated by Bull (1938). Subsequent studies by Houston (1957), Baggerman (1957, 1959, 1960a, b), and McInerney (1964) demonstrated a similar discriminatory capacity for juveniles of all five North American species of Pacific salmon and concerned selective responses of such fish presented with a choice of salinities.

Interpretation of the salmon studies has been hampered by the use of a test apparatus in which the fish were given only a limited number of salinities (usually 2) from which to choose and in which the salinity changes were abrupt. As a result of these technical difficulties, it was not possible to define the preferred salinity for juvenile salmon or to detect gradual changes in response. These problems have been largely overcome in the present study through the use of a shallow, continuous salinity gradient.

---

[1]Present address: Department of Zoology, Arizona State University, Tempe, Ariz., USA.

Printed in Canada (J1580)

MATERIALS AND METHODS

All fish were collected from the upper regions of the Cowichan River, Vancouver Island, B.C. Aerated fry cans were used to transport the fish to holding facilities at the Fisheries Research Board of Canada Biological Station, Nanaimo, B.C.

Test fish were acclimated to the experimental temperature of 10 C prior to use. The fish were never exposed to salt water during holding or acclimation.

Salinity preference was determined in a horizontal gradient. Ten series of tests were performed during the period from May 1967 to February 1968. Each test consisted of the evaluation of the responses of five fish individually, to a particular gradient.

Apparatus

The gradient apparatus consisted of a plexiglass trough, 6 ft in length and divided into 16 interconnected compartments by baffles extending one-quarter of the way up from the bottom and an equal distance down from the top (Fig. 1 and 2). Sheets of plastic screening were fixed horizontally to both the top and bottom rows of baffles, leaving an unobstructed observation area in the center. The sides and bottom of the trough were painted flat black and insulated with the exception of a narrow viewing slit that ran the length of the trough and was covered with a plastic, half-silvered mirror (one-way). Light from a single fluorescent tube, equal in length

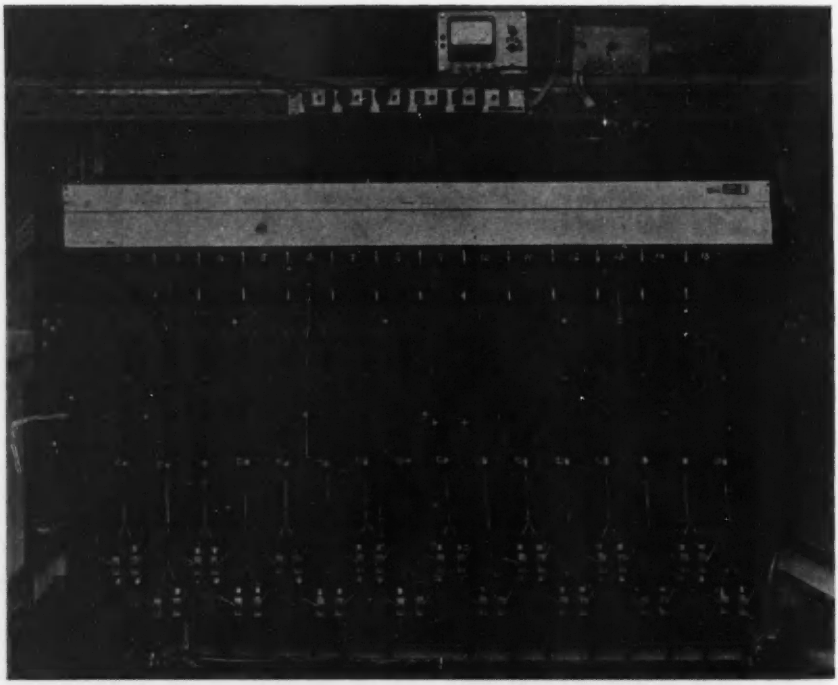


Fig. 1. The salinity preference apparatus. The large tanks at the top rear are fresh- and salt-water reservoirs. The pumps and insulated carboy at the center top are part of the refrigeration system. The gradient trough (16 compartments, including two entry ports) is in the center. Fresh and salt water are mixed at the Y-joints then passed up through flowmeters immediately below the trough into the bottom of each chamber. Salinity samples are taken from the rubber tubes below the trough. These tubes connect directly to overflow ports at the rear of the trough.

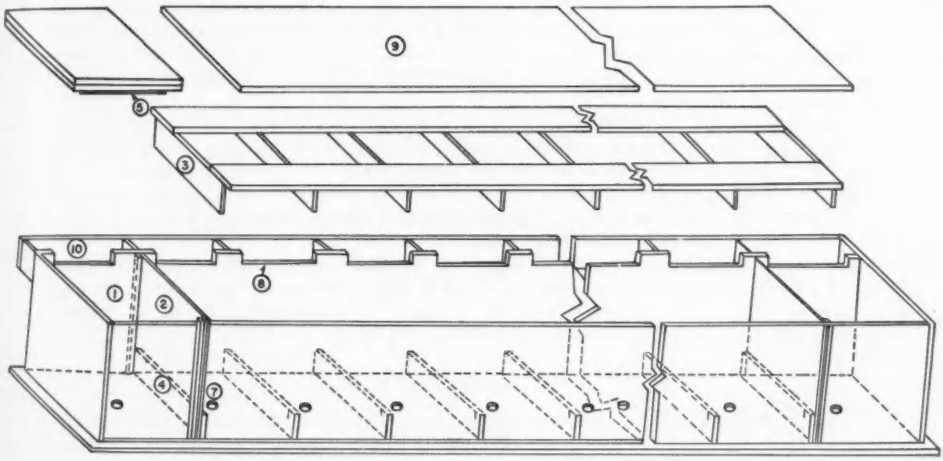


FIG. 2. Schematic exploded view of a portion of the gradient trough: (1) compartment 1, entry port; (2) removable partition; (3) top baffle; (4) bottom baffle; (5) cover for entry port; (7) water input; (8) overflow port; (9) translucent top plate; (10) drainage trough to sampling point. Light and plastic screen covering baffles are not shown.

to the trough, was diffused through a sheet of frosted plexiglass and confined to the top of the trough by a metal shade.

To avoid a possible bias in the results by differences in light intensity from one end of the trough to the other, the fluorescent tube was mounted slightly behind the trough, thus producing a very sharp front-to-back gradient. Any orientation by the fish to light intensity would, therefore, be expected to be from front to back rather than from end to end in the trough. The entire apparatus and observation area were located in a light-proof enclosure.

Fresh and salt water were gravity-fed from reservoirs to manifolds below the gradient trough and thence up through stopcocks to Y-joints, where mixing occurred. From the Y-joints, water passed trough flowmeters before reaching nozzles in the bottom of each compartment. Within the trough, water moved upward between the baffles, through the observation section, and out exit ports in the top rear of each compartment. The amount of mixing that occurred between compartments was negligible for gradients of the magnitude used.

PROCEDURE

The apparatus was first set up with a flow of fresh water to all compartments. A single fish was selected at random from the stock tank and introduced to the trough. Since the time required for fish to adjust to the new surroundings varied considerably, each was held in the gradient trough overnight prior to testing.

After the initial adjustment period, each fish was observed in a freshwater control situation and twice in each of two salinity gradients. The tabulation procedure was the same in all tests and consisted of recording the compartment occupied by the fish at 5-sec intervals for a 30-min period. The control test, in which the fish was observed in fresh water, was performed first. Then the stopcocks were adjusted to establish a salinity gradient increasing from fresh water at the observers left to approximately 13‰ at the right (gradient A). After 1 hr elapsed for the gradient to stabilize and for the fish to explore the new situation, the response of the fish to the gradient was recorded. The gradient was then reversed so that the freshwater portion was at the observer's right (gradient A′). A further 1-hr adjustment period was allowed followed by a 30-min period of observation.

The gradient was then adjusted to a minimum salinity of approximately 4‰ at the left end of the trough and 19‰ at the right (gradient B), a change of both position and magnitude. Adjustment period, observation of response, and reversal of the gradient (gradient B′) were exactly the same as described for gradients A and A′. The fish was then removed from the trough, measured, and discarded.

Salinities within the various compartments as determined immediately prior to each series of observations were compared with the frequency distributions of positions to determine the response to salinity. Temperature in each compartment was also checked immediately prior to each series of observations to ensure that no thermal gradient was present. Gradients of dissolved gas were unavoidable because of differences in solubility as related to salinity.

The response distributions for opposing gradient configurations (A–A′, B–B′) in each series were grouped, giving a total of 10 tests for each of the primary gradient situations (fresh water to 13‰, 4–19‰). In this way, any persistent biasing of the distribution as a result of tank features or ancillary factors was neutralized. The total number of observations in each of the primary gradient situations for each test series was therefore: 3600(5 × 2 × 360).

RESULTS

Fish in fresh water typically swam slowly from end to end of the trough, occasionally settling to the bottom screen or pausing to investigate air bubbles or detritus in the water. They seldom made a complete turn (180°) other than in the end compartments. The fish were almost continuously active with the exception of occasional brief periods of orientation to inflowing water or miscellaneous tank features.

The response to the sharp, front-to-back light gradient in the trough was variable. Most fish swam freely in the brighter front portion of the trough; however, any disturbance resulted in an immediate shift to the darkened rear area and a marked reduction in activity. Larger fish, particularly those approaching the smolt transformation, were more easily startled and tended to remain in the shaded area of the trough.

Trough dimensions had only a slight influence on the distribution of response. Fish in the control tests were found at about a 2% higher frequency in the center compartments than at the ends of the trough. This slight bias can be attributed to the tendency for the fish to occasionally reverse their direction prior to reaching the end compartments. The fish also showed a slight degree of orientation to the point of entry, even after the 12-hr adjustment period. A similar retention of spatial relations was observed by Houston (1957) for juvenile coho that were offered a choice of two compartments in a partially divided aquarium.

The introduction of a salinity gradient to the trough resulted in an immediate response by the fish. The turning frequency increased and the fish exhibited a mild alarm reaction. They quickly resumed their normal behaviour and the 1-hr adjustment period appeared to be ample for recovery from any fright.

During the period from June to December, the response to a gradient extending from fresh water to about 13‰ was bimodal in character with zones of preference occurring in fresh water and at 4–5‰ (Fig. 3, A–A′). Such a bimodal distribution of preferred salinities suggests that at least two factors underlie the preference response during this period. In January, the response

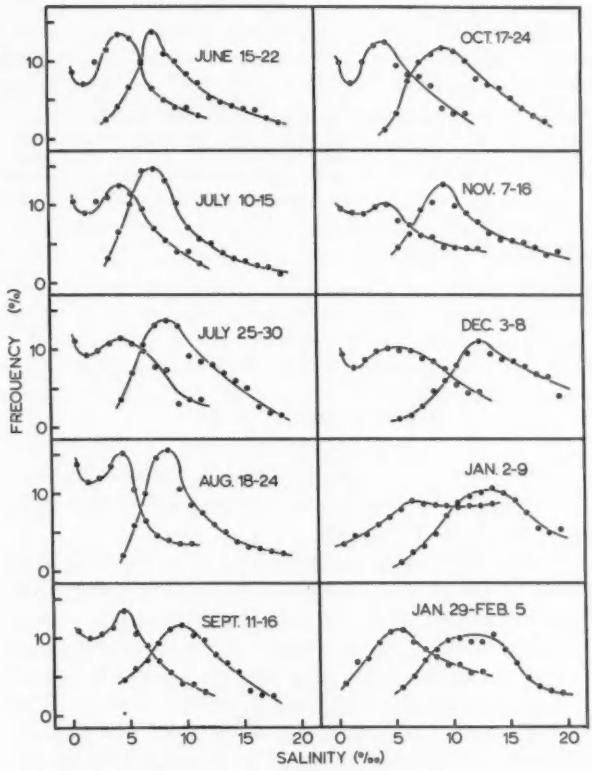


FIG. 3. Temporal changes in the salinity preference of pre-smolt coho salmon as determined in a horizontal gradient. Each pair of curves represents observations of five fish tested in two gradient situations. Gradient A–A′ (open circles) extended from fresh water to about 13‰; gradient B–B′ (solid circles) extended from about 4 to 20‰.

to fresh water declined sharply in intensity and the preference distribution became unimodal. Concomitant with the reduction in preference for fresh water, the remaining mode shifted to approximately 6–7‰. Of the two response peaks, the freshwater mode was consistently the smaller.

The response to a gradient from 3–4‰ to 18–20‰ was unimodal throughout the experimental period (Fig. 3, B–B′). The preferred salinity increased from 7–8‰ in June to 13–14‰ in February.

Both the decline in the preference for fresh water and the shift in position of the second mode in the response to gradient A–A′ corresponded with the marked increase in salinity tolerance (Fig. 4, broken line) found by Otto (MS, 1968) in juvenile coho in the period from October to December.

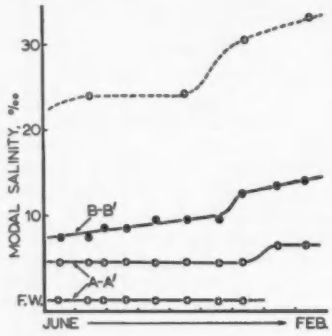


FIG. 4. Chronological changes in position of the various preference modes during the tests, and (broken line) changes in the upper incipient lethal salinity (that salinity at which 50% of the animals tested were calculated to survive the effects of salinity stress indefinitely) in the same period (from Otto, MS, 1968).

DISCUSSION

The results presented here support two general hypotheses indicated in studies by Houston (1957), Baggerman (1957, 1959, 1960a, b), and McInerney (1964): that salinity preference is functionally related to the physiological state of the individual and that this response may act as an orientation mechanism during the seaward migration.

Survival studies (Otto, MS, 1968) conducted concurrently with the salinity preference studies showed that juvenile coho cannot move directly into the sea during most of the pre-smolt period. The significance of a preference for fresh water is, therefore, apparent when aspects of the coho life cycle are considered. After emergence of fry from the redd, a partitioning of the year-class occurs according to the time of emigration from the parent stream. Two primary periods of emigration are recognized. The first occurs shortly after emergence and is believed to result from intraspecific competition for food and space (Chapman, 1962; Mason and Chapman, 1965). The second major emigration from the parent stream occurs at about age I, after the smolt transformation.

The fate of those fish that leave the stream prior to the smolt transformation is unknown at this time; however, it is clear that if they cannot avoid entering the sea, they must die. P. Wickett (personal communication) has suggested that, upon reaching the margin of the estuary, these fish either reverse their direction and attempt to swim back upstream or move along the shoreline to enter other streams if such are available. The freshwater preference observed during the period in which the pre-smolt emigration occurs is consistent with Wickett's observation.

With the approach of the smolt transformation, and the accompanying increase in salinity tolerance, preference for fresh water is greatly reduced. Since this reduction occurred considerably prior to the migratory period, it appears that the loss of a freshwater preference does not indicate the development of a "migratory disposition" as was suggested by Baggerman (1960b) but is instead an index of "migratory capacity."

The second preferendum, at salinities intermediate between fresh and sea water, is interpreted as selection of an optimum salinity concentration in relation to physiological state. The existence of such an optimum is supported by several lines of evidence. Otto (MS, 1968), in studying the effects of salinity on the growth of juvenile coho, demonstrated an optimal salinity corresponding closely with the second preferendum. In particular, it corresponded with the mode in the tests in which fresh water was excluded from the gradient. For example, during October and November the growth rate, food intake, and gross food conversion efficiency were all higher at 10‰ than for fresh water, 5, 15, 20, or 25‰ salinity. During this same period, the preferred salinity for gradient B–B′ was also at 10‰.

Further evidence of a salinity optimum in relation to physiological state was given by Rao (1968), who measured the oxygen consumption of rainbow trout (*Salmo gairdneri*) at various levels of activity and salinity. At all levels of activity, oxygen consumption was minimal at 7.5‰ relative to fresh water, 15, and 30‰ salinity.

Differences in position of the secondary preferred salinity in the two test gradients are interpreted as an apparatus effect. Since the two zones of preference (fresh water and an intermediate salinity) were located at different positions in the test apparatus the directive factors that underlie these preferenda are potentially competitive. Under natural conditions, the change in salinity per unit distance is typically much smaller than that exsiting in the test apparatus. Fish in the gradient apparatus were exposed to both of the preferred regions in close proximity, creating a situation of competition between directive factors. The vector resultant of such interaction would be expected to draw the adjacent boundaries of the two preferred zones together and disguise somewhat the lesser preferred region that lies between. Thus, in the test gradient that originated in fresh water and included both of the preferred zones, the position of the secondary preferendum presumably underestimates the true value. The value determined in the test gradient that did not include the freshwater preferred zone should not be influenced by such competitive interaction and should represent a more accurate estimate of the physiological optimum.

In considering the possible role of salinity preference as a directive mechanism during seaward migration, development of a preference for sea water has been pictured as a biphasic phenomenon (McInerney, 1964). In the initial portion of the sequence, a gradual increase in the preferred concentration occurs independently of any exposure to sea water. This gradual increase serves to direct the fish from the stream into the estuary, where a period of exposure to dilute salinities then initiates further changes, terminating in a preference for water of open ocean concentration.

For coho salmon, it is a gradual decline in the intensity of preference for fresh water that is of significance during the first portion of the sequence, a preference for intermediate salinities being present throughout the pre-smolt period. Since this decline occurs some time prior to the smolt transformation and seaward migration, other behavioural components (Hoar, 1958) must be

responsible for holding the fish within the stream during this period. The severance of these behavioural ties to the parent stream at the time of the smolt transformation should then be followed by a direct movement through the estuary to the physiological optimum salinity.

The increase in the preferred level from intermediate salinities to water of open ocean concentration as proposed by McInerney is not apparent from these experiments. Certainly the position of the physiologically determined preferendum would not increase beyond the isosmotic point (Otto, MS, 1968). It may be that as regulatory mechanisms become increasingly efficient as a result of age, increased size, or acclimatization by seawater exposure, the intensity of avoidance of salinities above the isosmotic point may be reduced to a level where salinity is of no significance as a directive or positioning factor. Other environmental components may then serve to direct the fish from the estuary into the open ocean.

ACKNOWLEDGMENTS

We acknowledge the advice and assistance of Dr D. F. Alderdice, Biological Station, Fisheries Research Board of Canada, Nanaimo, B.C., in whose laboratory the work was carried out. Others on the scientific and technical staffs of the Nanaimo station contributed in many ways to the completion of the study; especially, Mr F. P. J. Velson assisted in the laboratory work and his patience and hard work are acknowledged with thanks. Financial assistance from the Fisheries Research Board of Canada, the National Research Council (Grant A2069), and the University of Victoria is gratefully acknowledged. Dr S. D. Gerking, Arizona State University, reviewed the manuscript.

REFERENCES

BAGGERMAN, B. 1957. An experimental study of the timing of breeding and migration in the three-spine stickleback (*Gasterosteus aculeatus* L.). Arch. Neer. Zool. 12: 105–318.

1959. The role of external factors and hormones in migration of sticklebacks and juvenile salmon. *In* A. Gorbman [ed.] Symposium on comparative endocrinology. John Wiley and Sons, Inc., New York. 746 p.

1960a. Salinity preference, thyroid activity, and the seaward migration of four species of Pacific salmon (*Oncorhynchus*). J. Fish. Res. Bd. Canada 17: 295–322.

1960b. Factors in the diadromous migrations of fish. Symp. Zool. Soc. London 1: 33–60.

BULL, H. O. 1938. Studies on conditioned responses in fishes. 8. Discrimination of salinity changes in marine teleosts. Rep. Dove Marine Lab. Ser. 3, 5: 19–35.

CHAPMAN, D. W. 1962. Aggressive behaviour in juvenile coho salmon as a cause of emigration. J. Fish. Res. Bd. Canada 19: 1047–1080.

HOAR, W. S. 1958. The evolution of migratory behaviour among the juvenile salmon of the genus *Oncorhynchus*. J. Fish. Res. Bd. Canada 15: 391–428.

HOUSTON, A. H. 1957. Responses of juvenile chum, pink, and coho salmon to sharp sea-water gradients. Can. J. Zool. 35: 371–383.

MASON, J. C., AND D. W. CHAPMAN. 1965. Significance of early emergence, environmental rearing capacity, and behavioural ecology of juvenile coho salmon in stress channels. J. Fish. Res. Bd. Canada 22: 173–190.

MCINERNEY, J. E. 1964. Salinity preference: an orientation mechanism in salmon migration. J. Fish. Res. Bd. Canada 21: 995–1017.

OTTO, R. G. MS, 1968. Survival, preference, blood ion regulation, and growth of fresh water adapted pre-smolt coho salmon (*Oncorhynchus kisutch*) exposed to various salinities. M.Sc. Thesis. Department of Biology, University of Victoria, Victoria, B.C.

RAO, G. M. M. 1968. Oxygen consumption of rainbow trout (*Salmo gairdneri*) in relation to activity and salinity. Can J. Zool. 46: 781–786.

NOTES

Glucose Levels in Fresh *Tilapia* Muscle

A. HOFFMAN, J. G. DISNEY, B. E. GRIMWOOD, AND N. R. JONES

Tropical Products Institute
56/62, Gray's Inn Road, London, W.C.1, England

HOFFMAN, A., J. G. DISNEY, B. E.G RIMWOOD, AND N. R. JONES. 1970. Glucose levels in fresh *Tilapia* muscle. J. Fish. Res. Bd. Canada 27: 801–803.

The glucose content of *Tilapia* muscle from fish of different physiological condition was estimated at the time of death. Glucose was found to be present in all instances but the amount was considerably greater in rested aquarium fish than in exercised pond-reared fish.

Received December 16, 1969

ALTHOUGH IT HAS BEEN REPORTED (e.g., Jones, 1958; Burt, 1961) that glucose can be present as free sugar in muscle from freshly killed fish, it has been suggested that the sugar may be the product of early postmortem glycolysis and not necessarily present in the living tissue in significant amounts (Tarr, 1966). Consequently "zero time" control data in a number of reports concerning changes in concentration during storage could be in error although small time lags are hardly likely to be relevant to any commercial operation. Reported levels for glucose in rested aquarium cod (Burt, 1961), trawled codling (Jones, 1958), rainbow trout (Tarr and Leroux, 1962), and herring (Hughes, 1964) fall within the range 0.3–2.2 μmole per gram of tissue. The present report concerns glucose levels in the muscle of the tropical freshwater fish *Tilapia nilotica*. Fish of different physiological and nutritional conditions were analysed. The effect of different extraction and assay techniques was also investigated.

Materials and methods — Twelve *T. nilotica*, which were reared in ponds in Uganda and weighed approximately 1 lb, were caught by seine net. Six of these were dropped quickly into liquid nitrogen to represent "unexercised" control samples. The remaining six fish were transferred to a large tank and "exhausted" by beating the water until this evoked no response. The fish were then removed and killed by immersion as above. The use of liquid nitrogen should prevent any postmortem degradation of glycogen and all samples were stored subsequently in solid carbon dioxide.

Ten aquarium-reared *T. nilotica*, weighing approximately 150 g, were also used. These were removed with as little stimulation as possible and killed by immersion in liquid nitrogen. Eight of these fish had been well fed, but the remaining two had been without food for a period of 5 weeks.

For the estimation of glucose two forms of extraction were used: (1) neutralised perchloric acid extracts (Jones and Murray, 1961); and (2) extraction into 80% ethanol followed by partition with chloroform (Jones, 1958).

Printed in Canada (J1675)

The glucose content of these extracts was measured, on the day of preparation, by two enzymatic methods: (1) hexokinase/glucose-6-phosphate dehydrogenase (Slein, 1963); and (2) glucose oxidase/peroxidase (Huggett and Nixon, 1957, as modified by Burt, 1961).

Glycogen was also estimated in the two starved aquarium fish and in two of the well-fed fish. After extraction in potassium hydroxide, glycogen was estimated colorimetrically with anthrone reagent (Mokrasche, 1954).

Results and discussion — The results are summarised in Tables 1 and 2 where each figure represents an average of two determinations. The glucose concentration as estimated for the two extraction procedures by the two

TABLE 1. Glucose levels in pond-reared *Tilapia* (μmole/g).

	Perchloric acid extraction		Ethanol extraction	
	Method 1	Method 2	Method 1	Method 2
T. nilotica — pond fish "unexercised" before death	0.1	0.1	0.3	0.1
	0.8	1.0	0.9	0.8
	0.4	0.4	0.4	0.3
	1.4	1.0	1.3	1.4
	0.9	0.9	0.9	0.7
	1.2	1.4	1.3	1.1
T. nilotica — pond fish "exhausted" before death	0.6	0.6	0.5	0.6
	1.3	1.1	1.0	1.0
	1.0	1.2	1.8	0.9
	1.5	1.6	1.1	1.1
	0.9	1.1	0.9	0.9
	1.6	1.5	1.4	1.3

TABLE 2. Glucose levels in aquarium-reared *Tilapia* (perchloric acid extraction — method 2).

Well-fed		Starved	
Glucose (*μmole/g*)	Glycogen (*mg/100 g*)	Glucose (*μmole/g*)	Glycogen (*mg/100 g*)
3.6	447	3.1	230
3.0	428	2.9	278
3.0			
1.7			
3.3			
3.5			
3.9			
3.6			

methods of estimation agreed reasonably well and the levels of glucose found were in the range 0.1–3.9 μmoles/g. Since the fish were frozen alive in liquid nitrogen the results indicate in vivo levels in this species. Tarr (1954), using

liquid nitrogen freezing, indicated that glucose was probably absent from the muscle of living fish; but later he (Tarr, 1966) suggested that the methods used may have missed small concentrations of glucose. Other workers referring to glucose levels have quoted values, at or near death, of 0.3–2.2 μmoles/g for a variety of species and sampling techniques some of which may have permitted some degradation of glycogen. In the present study the samples were all frozen alive and the presence of glucose in fish muscle is now verified, although these techniques do not preclude some stimulation during freezing.

Apparently there is no clear difference between the glucose levels for the unexercised and the exhausted fish. However, the well-fed aquarium fish have considerably higher levels of glucose than either group of pond-reared fish. The degree of struggling in the net during catching was negligible in the case of aquarium fish, but some struggling during capture in the seine net was unavoidable. Thus the two groups of pond fish approximate the extreme conditions likely to be experienced in commercial fishing. Burt (1961) found, in cod, that rested muscle contained considerably more free glucose than exhausted muscle and the present results appear to substantiate his findings.

In the two starved fish the glucose level was of the same order as that in the well-fed fish. Thus starvation of this species does not appear to affect the level of muscle glucose at death. However, the glycogen content had fallen by only 40% during 5 weeks starvation despite a 6.3% loss in body weight. Consequently the effect of starvation requires further investigation.

Although the present results are confined to live fish frozen in liquid nitrogen, further work on *T. nilotica* is in progress and the pattern of postmortem glycolysis and its relation to the preservation of freshwater fish is being investigated.

REFERENCES

BURT, J. R. 1961. Free sugars and sugar phosphates in muscle of chill stored aquarium cod (*Gadus callarias*). J. Food Sci. 26: 462–468.

HUGGETT, A. ST. G., AND D. A. NIXON. 1957. Enzymic determination of blood glucose. Biochem. J. 66: 12 p.

HUGHES, R. B. 1964. Chemical studies on the herring (*Clupea harengus*). X. Histidine and free sugars in herring flesh. J. Sci. Food Agr. 15: 293–299.

JONES, N. R. 1958. Free sugars in chill-stored trawled codling (*Gadus callarias*) muscle. J. Sci. Food Agr. 9: 672–677.

JONES, N. R., AND J. MURRAY. 1961. Nucleotide concentration in codling (*Gadus callarias*) muscle passing through rigor mortis at 0°C. Z. Vergl. Physiol. 44: 174–183.

MOKRASCHE, L. C. 1954. Analysis of hexose phosphates and sugar mixtures with the anthrone reagent. J. Biol. Chem. 208: 55–59.

SLEIN, M. W. 1963. D-Glucose. Determination with hexokinase and glucose-6-phosphate dehydrogenase, p. 117–123. *In* H. U. Bergmeyer [ed.] Methods of enzymatic analysis. Academic Press, New York, N.Y.

TARR, H. L. A. 1966. Post-mortem changes in glycogen, nucleotides, sugar phosphates and sugars in fish muscles — a review. J. Food Sci. 31: 846–854.

TARR, H. L. A., AND M. LEROUX. 1962. Acid soluble phosphorus compounds and free sugars in fish muscle and their origin. Can. J. Biochem. Physiol. 40: 571–589.

A Record of the Hatchetfish *Sternoptyx diaphana* from the Grand Bank

T. K. PITT AND G. H. WINTERS

Fisheries Research Board of Canada
Biological Station, St. John's, Nfld.

PITT, T. K., AND G. H. WINTERS. 1970. A record of the hatchetfish *Sternoptyx diaphana* from the Grand Bank. J. Fish. Res. Bd. Canada 27: 804–805.

A single specimen of the hatchetfish *Sternoptyx diaphana* Hermann was caught at the surface on the southeast part of the Grand Bank. The most northerly records for the North Atlantic are from locations east of the Grand Bank in the Gulf Stream.

Received December 30, 1969

THE MOST NORTHERLY RECORDS of *Sternoptyx diaphana* Hermann known to us were reported by Murray and Hjort (1912) at 48°24′N and 36°53′W approximately 300 miles east of the Grand Bank, and a single specimen by Koefoed (1962) at 48°02′N and 39°55′W about 120 miles closer to the bank but well into the Gulf Stream. Schultz (1961, 1964) lists a specimen from just south of La Have Bank, 42°46′N, 63°22′W as a northerly record for the western North Atlantic. Halkett (1913) lists *S. diaphana* as occurring from the Grand Bank southward to Santa Cruz Island. The Grand Bank report apparently originated with Goode and Bean (1896) when the latter reported that a schooner took a single specimen (Cat. No. 26235 USNM) "off the Grand Bank." The exact position was apparently not reported.

The specimen (Fig. 1) reported here was caught June 3, 1969, by the Fisheries Research Board of Canada's research vessel *A. T. Cameron* on the surface in a neuston net at 44°30′N, 48°48′W. The surface temperature was 3.6 C and the salinity 33.4‰. The fish was alive when caught.

The following measurements (preserved specimen) were recorded: length, snout to caudal base 21 mm; postorbital length 14 mm; greatest diameter of the eye 3.5 mm.

Various meristic counts were recorded and except for the number of normal gill rakers on the lower arch (4) these were all within the range given by Schultz (1961, 1964) for Atlantic specimens.

According to most authors this species is usually taken at 100–1000 fath (183–1830 m), although Goode and Bean (1896) state that it is sometimes taken at the surface especially at night as was this specimen. Apparently it is usually found in warmer water than the 3.6 C recorded here and was probably transported from the Gulf Stream to the Grand Bank by surface currents.

REFERENCES

GOODE, G. B., AND T. H. BEAN. 1896. Oceanic ichthyology. Deep sea and pelagic fishes of the world. U.S. Nat. Mus. Spec. Bull. 2: 553 p.

Printed in Canada (J1684)

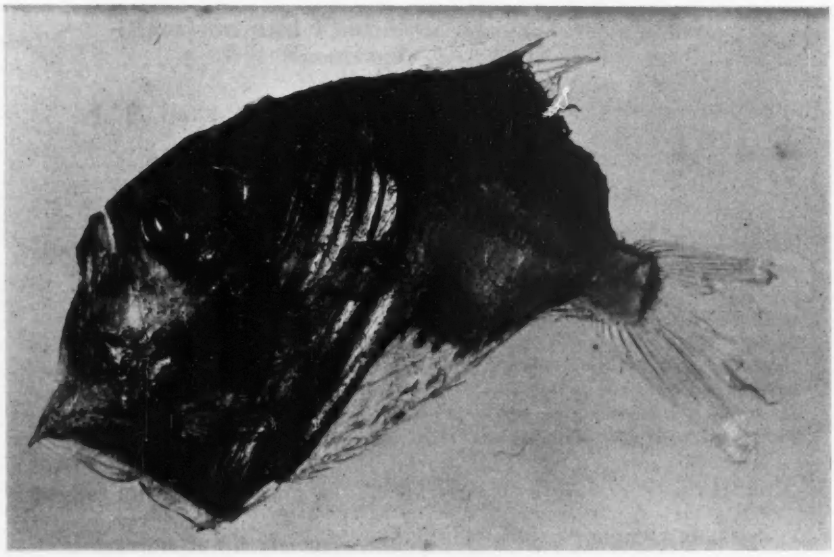


FIG. 1. *Sternoptyx diaphana* caught at the surface on the southeast part of the Grand Bank (approximately ×5).

Pitt and Winters — J. Fish. Res. Bd. Canada

HALKETT, A. 1913. Check list of the fishes of the Dominion of Canada and Newfoundland. King's Printer, Ottawa, Ont. 138 p.

KOEFOED, E. 1962. Isospondyli 2, Heterophotodermi 3. Michael Sars North Atlantic deep-sea expedition 1910. Vol. IV, Pt. II: 1–14.

MURRAY, J., AND J. HJORT. 1912. The depths of the ocean. Macmillan, and Company, Limited, London. 821 p.

SCHULTZ, L. P. 1961. Revision of the marine silver hatchetfishes (Family Sternoptychidae). Proc. U.S. Nat. Mus. 112: 587–649.
1964. Family Sternoptychidae. *In* Fishes of the western North Atlantic. Mem. Sears Found. Mar. Res. 4: 241–272.

Mercury Determination in Fish Samples by Wet Digestion and Flameless Atomic Absorption Spectrophotometry

J. F. UTHE, F. A. J. ARMSTRONG, AND M. P. STAINTON

Fisheries Research Board of Canada
Freshwater Institute, Winnipeg 19, Man.

UTHE, J. F., F. A. J. ARMSTRONG, AND M. P. STAINTON. 1970. Mercury determination in fish samples by wet digestion and flameless atomic absorption spectrophotometry. J. Fish. Res. Bd. Canada 27: 805–811.

A simple, rapid method is given for determination in fish of mercury at concentrations measured in parts per million (ppm). Samples are digested at 50–60 C with sulphuric acid and oxidised with permanganate. Mercury in the digest is determined by atomic absorption spectrophotometry. Recovery of mercury added to samples was complete, with good precision. Standard deviations from triplicate analyses were ±0.039 at the 0.1 ppm level and ±0.051 at the 0.5 ppm level. In a day, three workers analysed 15–20 samples in triplicate.

Received February 16, 1970

DETERMINATION OF the mercury content in fish tissue at the level of parts per billion has recently become very important in western Canada (Wobeser, MS, 1970). This has commonly been done by one of three different methods (Smart, 1968). Chemical oxidation of the sample followed by dithizone extraction is common but appears to require much time, complex digestion equipment, and many manipulative steps. Schöniger flask combustion has been used but suffers from the above-mentioned drawbacks as well as showing relatively low recoveries upon spiking. Neutron activation analysis is extremely sensitive and accurate but requires much time and sophisticated equipment. In this preliminary communication we describe the method for determining mercury at the residue level, which we developed in this laboratory. It has the advantage of: (1) use of simple digestion equipment allowing many samples to be processed concurrently, without attention; (2) short analysis time allowing a large number of samples to be processed per day; (3) small sample size and

Printed in Canada (J1719)

reagent volumes helping to eliminate large reagent blank values; and (4) accuracy and precision as indicated by analysis of spiked samples and standard samples.

WET DIGESTION

Temperatures must be kept low and oxidising conditions used to avoid loss of mercury. The oxidation procedure used here is essentially that of Jacobs et al. (1960) as modified by R. H. Dunlop (personal communication).

Procedure

(1) Place 0.1- to 0.5-g sample in the bottom of a 30-cc Kjeldahl flask, using the device shown in Fig. 1 to ensure that no sample is above the level of the acid during the solubilization step.

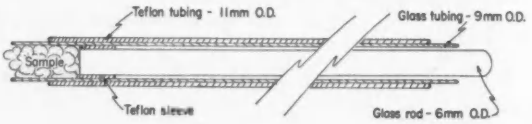


FIG. 1. Device for placing sample at bottom of Kjeldahl flask. O.D. = outside diameter.

(2) Add 4.0 cc sulphuric acid (sp gr 1.84).

(3) Incubate flasks in a shaking water bath at 50–60 C until a clear solution is obtained. With fish samples the solution is a deep red–brown, and an intense light source (e.g., a 150-w spotlight covered with aluminum foil with a small hole in it) is useful for checking the dissolution.

(4) Remove samples from bath, allow to stand for 1 hr, cool in ice.

(5) Add 15 cc 6% w/v potassium permanganate solution, slowly, with swirling. Allow to stand 30 min at room temperature.

(6) Close flask with polyethylene stopper. Samples at this stage may be kept for at least a day before analysis without apparent loss of mercury.

Observations

A source of trouble in the oxidation with permanganate is the use of too vigorous conditions during the acid dissolution. Too much heating can cause carbonization, after which samples cannot be completely oxidized by permanganate. The properly digested mixture contains much suspended material, but this is mostly hydrated oxides of manganese, which should dissolve completely, to give a colourless clear or slightly opalescent solution in the last stage of the determination when the reductant is added.

ATOMIC ABSORPTION SPECTROPHOTOMETRIC METHOD FOR MERCURY

Flameless atomic absorption methods for determination of mercury are sensitive and rapid by reason of the very high absorbance at 253.7 nm of mercury vapour, and the ready volatilization of the metal from acid-reducing solutions at room temperature. The procedure adopted in this work has been derived from that of Hatch and Ott (1968) with a change in the system for transfer of mercury vapour, which has given increased sensitivity, speed, and reproducibility.

Experimental

Hatch and Ott (1968) reduced mercury in acid solution with a sodium chloride/hydroxylamine sulphate/stannous sulphate reagent mixture and aerated the sample in a recirculation system. Air was passed through the reaction mixture, a drier, and the spectrophotometer cuvette back to the reaction flask. Absorbance of mercury vapour increased to a constant value in 3 min. We have used this procedure but have had difficulty in getting constant readings. Absorbance has usually decreased rapidly within seconds of reaching a maximum, as if mercury was being lost from the system. We have not traced this loss but think that oxidation or adsorption of mercury vapour may be the cause. As a result, the sensitivity of the method is very dependent on pump speed, and is difficult to reproduce. Furthermore, we have not been able to avoid some loss of mercury to the air when handling solutions containing hydroxylamine sulphate and transferring them to the apparatus, and further losses on addition of stannous sulphate, unless this was done very quickly and the apparatus closed promptly. We have therefore devised a closed reaction chamber in which reducing agents can be added without losing mercury vapour, and for which the time of transit and dilution of the vapour mixture is decreased. We have somewhat increased the amounts of reducing agents added to take care of excess oxidant left after digestion. With this apparatus, mercury vapour is partially equilibrated between the solution and the air above it before this air is swept out through a drier to the cuvette.

Atomic absorption spectrophotometer — Perkin-Elmer (P.E.) model 303 with automatic null recorder readout (303–0103) or model 403. Other atomic absorption spectrophotometers could be used if they allow mounting a flow-through cuvette in the light path, and it has also been possible to use a Beckman DK2 recording spectrophotometer by replacing the tungsten lamp with a small mercury vapour lamp (P.E. 303–0703 with its proper power supply).

Lamp — Either of the Perkin Elmer instruments may be used with a hollow-cathode mercury lamp 303–6044 *or* a mercury lamp 303–0703 and discharge lamp power supply.

Chart recorder — 10-mv chart recorder, chart speed 8–12 inches/hr.

Flow-through cuvette — Borosilicate glass, with silica end windows 22-mm diameter, 1-mm thickness, attached with epoxy cement. It is also possible to use standard silica 100 × 22-mm cylindrical absorptiometer cuvettes with two filling tubes.

Reaction apparatus — Borosilicate glass with polytetrafluorethylene stopcocks as shown in Fig. 2.

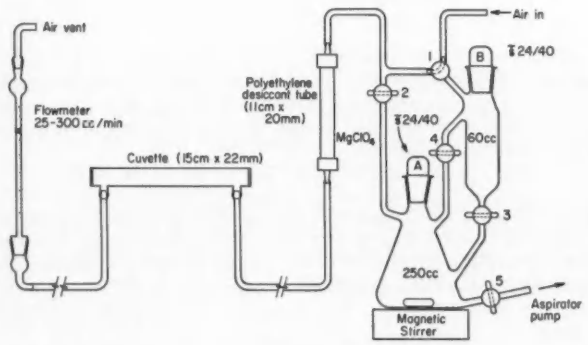


FIG. 2. Apparatus for volatilisation of mercury. Cuvette is mounted in light path of atomic absorption instrument for absorbance measurement at 253.7 nm.

Also required — Magnetic stirrer, air regulator, flowmeter (25–300 cc/min), stopwatch, compressed air supply, aspirator pump.

REAGENTS

Reductant —	Water	About 600 cc
	H_2SO_4 (sp gr 1.84)	100 cc
	NaCl	5 g
	$(NH_2OH)_2 \cdot H_2SO_4$	10 g
	$SnSO_4$	20 g

Prepare in order given, cool, make to 1000 cc, mix, filter.

Mercuric chloride standard A — Dissolve 0.1354 g $HgCl_2$ in water, make to 100 cc in graduated flask. 1.00 cc = 1.00 mg Hg. Stable 1 month.

Mercuric chloride standard B — Dilute 1.00 cc standard solution A above, to 100 cc, in graduated flask, using approximately 1 N H_2SO_4. 1.00 cc = 10.0 μg Hg. Stable 1 day.

PROCEDURE

(1) Set up atomic absorption spectrophotometer with mercury lamp, and with flow-through cuvette in place of burner:

Wavelength	253.7 nm
Slit setting	3
Scale	3×

Adjust wavelength for maximum energy; align cuvette for minimum absorbance.

(2) Have apparatus empty and clean, with taps in positions shown in Fig. 2, magnetic stirrer off, air flow 250 cc/min.

(3) The sample is received in 30-cc Kjeldahl flask, and contains sulphuric acid, hydrated oxides of manganese, and possibly some permanganate, in a volume of about 20 cc. It is important to transfer all of the solid material with the sample.

(4) Place 50 cc reductant in a graduated cylinder. Place sample in flask, and replace stopper A. Rinse Kjeldahl flask with two 10-cc portions of the reductant and place rinsings and remainder of reductant into funnel. Replace stopper B. Start magnetic stirrer.

(5) Open taps 4 and 3 to add reductant to sample. Start stopwatch, close taps 4 and 3.

(6) After 60 ± 2 sec, open taps 2 and 3, and turn tap 1 120° clockwise to divert air stream through funnel and flask.

(7) Note maximum pen deflection on chart; return taps to initial positions.

(8) Open stoppers A and B and tap 5 to empty the apparatus. Rinse funnel and flask twice with distilled water.

(9) Close tap 5, replace stoppers A and B, stop magnetic stirrer. Apparatus is ready for next sample.

BLANK DETERMINATION

Since the blank has components from the reagents used in the digestion, and also from those in the reductant, it may be necessary to determine them separately if for any reason the whole sample is not used. Normally the total blank is determined by carrying out the complete procedure of digestion and analysis with no sample.

CALIBRATION

A calibration curve should be prepared at least once daily or when operating conditions change. This is best done with 20-cc portions of 20% v/v H_2SO_4 in Kjeldahl flasks, to which known additions of mercury have been made. A set of six, from 0.00 to 0.25 μg Hg, is convenient, with mercuric chloride standard B added from a microburette.

RESULTS AND DISCUSSION

Figure 3 shows a calibration curve at 3× sensitivity for quantities of mercury up to 0.25 μg. On sample weights of 0.5 g these would represent up to 0.5 ppm. Instrument noise is usually less than 1 scale division. This

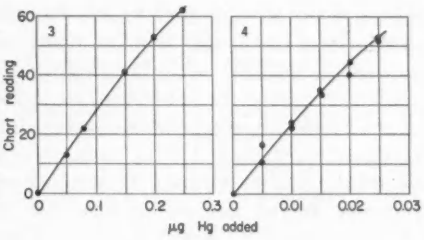


FIG. 3. Relations between chart reading and quantity of mercury added to reaction flask. Full scale chart reading, 100 units is 10-mv signal and represents absorbance 0.15. Sensitivity setting 3×, 60 sec stirring, air flow 250 cc/min.

FIG. 4. Relations between chart reading and quantity of mercury added, sensitivity setting 30×. Full scale, 100 units, represents absorbance 0.015.

is the system normally used; Fig. 4 shows the extent to which sensitivity can be pushed by simple amplification. There is a loss in reproducibility, as shown by the duplicate points, and noise increases markedly, at sensitivity setting 30×. Figure 5 shows the increase in absorbance with stirring time. Equilibrium

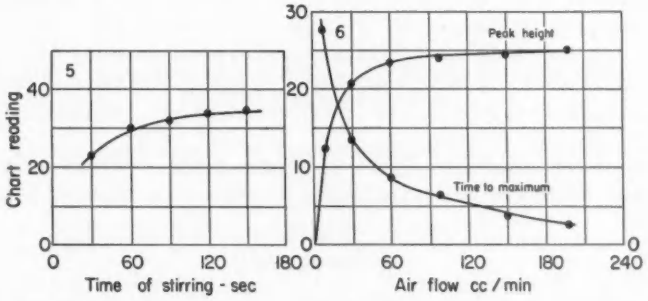


FIG. 5. Relations between chart reading and time of stirring before sweeping vapours from flask. Sensitivity 3×, 0.100 µg Hg, air flow 250 cc/min.

FIG. 6. Relations between peak height and air flow, and between time to reach maximum reading and air flow. Sensitivity 3×, 0.100 µg Hg, stirring time 60 sec.

appears to be approached in 150 sec, and 60 sec is not long enough, but was chosen as a compromise between sensitivity and speed. Figure 6 shows the relations between rate of air flow and sensitivity (peak height) and the time required to reach maximum reading. It is seen that neither is very sensitive to change at rates of air flow above 100 cc/min.

The standard deviation with known additions of mercury, *for the atomic absorption part* of the analysis only, and in different runs of analyses is ±0.002 at the 0.100-µg Hg level. The day-to-day reproducibility of calibration figures is satisfactory, the standard deviation being ±6 divisions at the 50-chart division level.

The sensitivity of the method depends of course on the relative volumes of sample plus reagents, air space in apparatus, volume and length of cuvette, rate of air flow, stirring time, reducing power of reagents, temperature, usable amplification and noise in the electronics, and maybe other factors. We have

been concerned here only to get enough sensitivity and precision for analysis up to about the 1-ppm level of mercury in fish samples. However, as a guide to what is possible with the method, for use in other applications we have tried to measure the molecular extinction coefficient of mercury vapour and to assess the partition between reagent solution and air. Experiments with cuvettes containing dry air saturated with mercury vapour at about 26 C gave values for ϵ varying between 2 and 4 $\times$ 10^6. The Beer-Lambert laws were not obeyed, low extinction values being obtained with long path lengths. This is probably a result of line broadening in the light source so that only the centre part of the line is absorbed (Barringer, 1966). By using dilute preparations of mercury vapour, with cuvettes of different lengths, we have obtained consistent values of $\epsilon = 4.1 \times 10^6$ mole^{-1} cm^{-1}, which we have used to calculate the concentration of mercury vapour in the cuvette when peak height is measured. Using the apparatus of Fig. 2 and a mercury addition of 0.100 μg in the flask, only 0.007 μg, or 7% of that added, is presented for atomic absorption measurement. We have also measured the concentrations of mercury in air above solutions containing known amounts of mercury in the mixture of acid and reductant. Distribution ratios so found came in the range 4.5–5.5 at 24 C, that is, the concentration in air at equilibrium is about one-fifth of that in the reagent.

These observations show that if it were required, a considerable increase in sensitivity could be got by modifying the relative volumes of parts of the apparatus.

OBSERVATIONS

It is possible to analyse 15–20 samples, in triplicate, per day, with three persons. Some selection of reagents for low blank may be necessary but we have had little difficulty when using analytical reagent quality materials. Normally blanks for the whole determination lie in the range 0.005–0.010 μg Hg.

RESULTS OF ANALYSIS

Samples of fish (walleye, *Stizostedion vitreum vitreum*) were spiked with known additions of mercury (as mercuric chloride) and analyses done in quintuplicate with the following results:

Mercury added (μg)	Mercury recovered (μg)
0.100	0.101±0.010
0.200	0.201±0.016
0.500	0.530±0.008

A sample of wheat flour (International Atomic Energy Agency Code 66/10) of accepted mercury content 4.7 $\pm$ 1.5 ppm gave 4.92 $\pm$ 0.45 ppm with this method. Analyses of fish here are usually done in triplicate. Examination of about 60 analyses, taken at random from records, has shown standard deviations of $\pm$0.039 at the 0.1 ppm level and $\pm$0.051 at the 0.5 ppm level. Interlaboratory comparisons are being made and will be reported later.

Acknowledgments — We thank J. D. Jamieson, B. W. Hauser, and H. Boese for comments and additional analyses.

REFERENCES

BARRINGER, A. R. 1966. Interference free spectrometer for high sensitivity Hg analysis of soils, rocks and air. Inst. Mining Met. Trans., Sect. B 75(74): 120–124. (Chem. Abstr. 66: 25718.)

HATCH, W. R., AND W. L. OTT. 1968. Determination of submicrogram quantities of mercury absorption spectrophotometry. Anal. Chem. 40: 2085–2087.

JACOBS, M. B., S. YAMAGUCHI, L. J. GOLDWATER, AND H. GILBERT. 1960. Determination of mercury in blood. Ind. Hyg. Ass. J. 21: 475–480.

SMART, N. A. 1968. Use and residues of mercury compounds in agriculture, p. 1–36. *In* F. A. Gunther [ed.] Residue reviews. Vol. 23. Springer-Verlag, New York.

WOBESER, G. MS, 1970. Mercury concentrations in muscle of fish from the Saskatchewan River. Paper presented to Canadian Committee on Freshwater Fisheries Research, meeting no. 3, January 1970, Ottawa, Canada.

World Distribution of Brown Trout, *Salmo trutta*: Further Observations

HUGH R. MACCRIMMON, T. LARRY MARSHALL, AND BARRA L. GOTS

Department of Zoology
University of Guelph, Guelph, Ont.

MACCRIMMON, H. R., T. L. MARSHALL, AND B. L. GOTS. 1970. World distribution of brown trout, *Salmo trutta*: further observations. J. Fish. Res. Bd. Canada 27: 811–818.

This note supplements the original paper by MacCrimmon and Marshall (J. Fish. Res. Bd. Canada 25: 2527–2548, 1968) in presenting modified and more complete information on the native range, attempted introductions, and present world distribution of the brown trout.

Received October 30, 1969

SINCE PUBLICATION of the world distribution of brown trout, *Salmo trutta* Linnaeus (MacCrimmon and Marshall, 1968), additional reference material and information have been acquired largely through personal communications arising from that paper. This note comprises additions and modifications to the previously published data on the native and present range of the species.

Native range — There is some question about the limits of the native range of the brown trout. However, there is reliable evidence that the pristine distribution extended southward from that previously given to include the tributaries of the Adriatic and Aegean seas in Yugoslavia, Albania, and Greece (Behnke, Livojević, and Pažur, personal communication: Serbétis, 1954). Also, to the east the native range would seem to have included the Timanskiy–Ural Mountain ranges in the USSR (Burmakin, 1963); and to the south the

Printed in Canada (J1640)

Oronte River in Lebanon (Hoyek, personal communication), the headwaters of the Tigris and Euphrates rivers and Caspian Sea tributaries in Turkey, and the Amu Dar'ya drainage of the Aral Sea through Afghanistan into West Pakistan (Behnke, personal communication).

The brown trout still occupies waters within all of the above-stated native range except in northern Africa, where it now occurs only in the Atlas Mountain range of Morocco (Morocco Ministry of Agriculture, 1960), and in the Volga River basin of the USSR (Nikol'skii, 1945) where it would seem to be now less widely distributed.

EURASIA

Union of Soviet Socialist Republics — The first known attempt to transplant *S. trutta* beyond its native range was in 1852 when, on the recommendation of K. M. Bera, seven plantings totalling 270,000 fish were made in Chudskoe Lake in the Baltic region without success (Burmakin, 1963). Of 22 additional plantings between 1852 and 1939, trout were established in four waters, namely, Arakul' Lake in the arctic region, Lake Vel'ye and Lake Ukleyno in the Ponto–Caspian–Aral region, and Losoenka Creek in the Baltic region. In addition, the species is now acclimated in the Ob' basin by the Ural Mountains (56°N, 61°E) and maintained by stocking in waters of the Krasnoyarsk District. Plantings of Lake Seven trout (*Salmo trutta ischchan*) into Lake Issyk Kul' beginning in 1930, and of Aral Sea trout (*Salmo trutta oxianus*) into the headwaters of the Syr Dar'ya River in 1953, have established fisheries in each of these waters.

West Pakistan — Further information on the brown trout in India and West Pakistan (Husain, personal communication) reveals that fish of English origin were established in ponds at Harwen in the Kashmir Valley about 1903, and first provided eggs for culture in 1906. The first brown trout hatchery was established in West Pakistan at Shinu (Kaghan Valley) with ova originally obtained from Harwen. Subsequent plantings in the nearby Kunhar River and tributaries resulted in thriving self-sustaining populations, particularly near Naran and including the popular Saif-ul-Malak Lake (alt 3050 m) (Husain, personal communication).

Plantings of Gilgit trout into Lutkoh River of Chitral in 1945 established a prosperous fishery and led to the construction of a hatchery in the nearby Bombrait Valley. Other recently developed brown trout hatcheries distributing trout to reservoirs and natural waters are near Madyan (Swat) and Quetta (Baluchistan), and a new facility has been sanctioned in Parachinar (Husain, personal communication). As a result of brown trout culture, substantial brown trout fisheries now occur in West Pakistan with the species thriving in streams above 1190 m in elevation (Doha, personal communication).

Népal — Brown trout from Kashmir were introduced into rearing facilities in Népal in March 1969, preliminary to an attempt to establish the species in natural waters (Woynarovich, personal communication).

Japan — An early reference (Worth, 1893) documents specifically the transfer of brown trout from North America to Tokyo in 1892–93, Professor C. Sasaki receiving 10,000 eggs of *Salmo levenensis* (Loch Leven trout) and 10,000 eggs of *Salmo fario* (German trout) from the U.S. Fish Commission. At present, brown trout are naturalized only in Chuzenji Lake, Nikko City, Tochigi Pref. (Shiraishi, personal communication).

NEW ZEALAND AND AUSTRALIA

New Zealand — The distribution of brown trout on the South Island is more extensive than implied in the discussion by MacCrimmon and Marshall, (1968, p. 2543), occurring over the island upwards from sea level (Allen, 1957; Scott, personal communication). However, on the North Island the species is absent from the Northland and Coromandel peninsulas, the tip of the East Cape region, and most of the east coast river systems except those entering Hawkes Bay (Allan, 1957).

Territory of Papua and New Guinea (Australia) — Rapson (personal communication) reports that the initial planting of brown trout fingerlings into tributaries of the Wahgi River near Nondugl and into a small pond on the Nondugl Station by Sir Edward Hallstrom in December 1949 established a population. Details are lacking on subsequent plantings made into the Asaro (Purari watershed), Gumanch (Wahgi–Purari watershed), and Baiyer (Sepik watershed) river systems (Rapson, personal communication). No captures of brown trout have been reported from the Asaro River since 1962, but trout are definitely known to breed successfully in the Gumanch and Baiyer systems (Conroy, personal communication).

Plantings of brown trout were made in the Baiyer and Gumanch rivers and in the Erave–Purari watershed (Maugani and Iaro rivers) in 1968. These fish, like those previously planted, were from Australian sources (Conroy, personal communication).

NORTH AMERICA

United States of America — Scrutiny of early Government records (U.S. Commissioner of Fish and Fisheries, 1889–91, 1892, 1894; Worth, 1893) has shown minor discrepancies between dates of first introduction to several states recorded there and in source materials used in the original paper. Table 1 lists those dates believed by the authors to be the most authentic for each state. However, there are no changes in the North American distribution from that reported previously.

Mexico — First attempts to introduce brown trout into Mexico occurred between 1891 and 1894 when eggs of German (Von Behr) brown trout were sent to the Government of Mexico by the U.S. Fish Commission. The 1894 shipment included, also, eggs of the Loch Leven variety (U.S. Commissioner of Fish and Fisheries, 1889–91, 1892, 1894). These and later plantings were

TABLE 1. Dates of first importations of brown trout in the United States of America.

State	Year of introduction	State	Year of introduction
Alabama	None	Montana	1889
Alaska	None	Nebraska	1889
Arizona	1891–92	Nevada	1895
Arkansas	1890	New Hampshire	1887
California	1892	New Jersey	1889–90
Colorado	1887	New Mexico	1893–94
Connecticut	1889–90	New York	1883
Delaware	1889–90	North Carolina	1887
Florida	1956–57	North Dakota	Unknown
Georgia	Unknown	Ohio	1890–91
Hawaii	1935	Oklahoma	1930's
Idaho	1892–93	Oregon	1897–98
Illinois	1887	Pennsylvania	1886
Indiana	1889–90	Rhode Island	1894–95
Iowa	1885	South Carolina	1887
Kansas	1890–91	South Dakota	1893–94
Kentucky	1891–92	Tennessee	Unknown
Louisiana	None	Texas	1892–93[a]
Maine	1885	Utah	1900
Maryland	1889–90	Vermont	1898–90
Massachusetts	1887	Virginia	1890–91
Michigan	1883	Washington	1935 and earlier
Minnesota	1888	West Virginia	1925–26
Mississippi	None	Wisconsin	1887
Missouri	1889–90	Wyoming	1889–90

[a]No survival.

apparently without success, and the species is still believed to be absent from the Country (Alvarez, 1950; Gandara, personal communication).

Jamaica — Brown trout fingerlings from New Jersey were introduced into a stream in the hills of the Parish of St. Thomas in July 1968. The fingerlings arrived in poor condition and there is no evidence of survival. However, as water temperatures seemed suitable, a second plant was contemplated for 1969 (Royer, personal communication).

AFRICA

Malawi — Although the authors reported that there was a naturalized population of brown trout in the Zomba Plateau (based on Harrison et al., 1963), these trout have been identified as *Salmo gairdneri*, which is the only species of salmonid in the country (Bowden, Crichton, and Fuller, personal communications).

Uganda — As previously reported, the original brown trout planting of 1930 was unsuccessful. There was, however, from 50,000 eggs, imported from England to a small hatchery on the River Mubuku in the Ruwenzori Mount

foothills in 1932, a population established in the River Ruimi at an altitude of 5110–1220 m. A similar planting in the River Mubuku was a failure as was a 1952 introduction into the Siti, off Mount Elgin (Biribonwoha, personal communication).

Democratic Republic of the Congo — An attempt to establish brown trout in Lac Kivu during 1957–58 proved to be a failure (Lumbala, personal communication).

Madagascar — Eggs of *Salmo fario* (la truite commune) first arrived at the Manjakatompo Station in the Ankaratra (alt 1900+ m) in 1926 and trout presently are naturalized in the mountain rivers above an elevation of 1700 m although captures are rare (Kiener, 1963).

Kerguelen Islands (49°S; 70°E) — Brown trout were imported, probably from France, around 1955 and presently the species is resident in three small natural waters (Vibert, personal communication).

SOUTH AMERICA

Bolivia — Brown trout of German origin were introduced into waters of the Tunari Range (Cordillera Oriental) in 1935 (Terrazas, personal communication) and, in 1939, were stocked in Lake Titicaca (Matsui, 1962). The species is now naturalized in the mountain waters of the Oriental and Real ranges, and hybridization with the rainbow trout occurs (Terrazas, personal communication).

Colombia — Brown trout of Loch Leven and Von Behr strains (10,000 of each) were sent to Colombia about 1892 by the U.S. Fish Commission (U.S. Commissioner of Fish and Fisheries, 1892). However, despite this and later attempts to introduce brown trout, the species has not prospered in Colombia (Velasquez, personal communication).

Ecuador — Brown trout, originally imported from Chile in 1950, 1957, and 1959 (totalling 90,000 eggs) and from the United States in 1954, have been planted in lakes and rivers of the Provinces of Azuay and Cañar not already inhabited by rainbow trout. The United States made further shipments of 100,000 eggs in 1967 and 350,000 eggs in 1968. Acclimation of the species in Ecuador has not yet been confirmed (Montenegro, personal communication).

Venezuela — Brown trout, along with rainbow and brook trout, were introduced into Venezuela in 1938 and, beginning in 1941, eggs were collected from naturalized stocks of trout (Leon, 1966). Trout fingerlings are now being planted in lakes and rivers of the region of the Andes Venezolanos (Cordillera de Mérida, provinces of Trujillo, Mérida, and Táchira) (Leon, 1966).

DISCUSSION

The degree of response to the brown trout distribution paper indicates a keener world interest in the species as a sport fish than previously indicated, especially in those geographic regions where water temper-

FIG. 1. Known world distribution of brown trout, *Salmo trutta*.

ature would seem, through latitude or elevation, suitable for survival of the species. New extensions in range reported in this note (e.g., West Pakistan, Madagascar, Uganda, and Bolivia) are further evidence of the adaptability of the brown trout to a variety of aquatic habitats.

A history of failure to establish brown trout in a country or geographic region should not discourage further attempts at naturalizing the species. There is considerable evidence that poor quality of stock, inferior culture procedures, and inadequate knowlege of brown trout ecology for effective plantings may each have contributed to failures in the past. Further, the presence of resident or naturalized populations may be unknown in the absence of anglers skilled in catching the species unless intensive inventories through electrofishing, or other means, are undertaken.

New details recorded in this note would seem to complete a valid description of the present world distribution of the brown trout (Fig. 1). The authors are indebted to many people, particularly those cited in the text, for making the revision possible.

REFERENCES

ALLEN, K. R. 1957. Natural areas in the distribution of freshwater fish. Proc. N.Z. Ecol. Soc. 4: 14–15.

ALVAREZ, J. 1950. Claves para la determinación de especies en los peces de las aquas continentales mexicanas. Secretar. Mar.; Dir. Gral. Pesca Ind. Con. 144 p.

BURMAKIN, E. V. 1963. Acclimatization of fresh water fishes in the USSR. Proc. State Sci. Res. Inst. Lake River Fish. 53: 317 p.

HARRISON, A. C., K. E. SHORTT-SMITH, J. H. YATES, R. A. JUBB, G. RUSHBY, AND C. T. FLAMWELL. 1963. Freshwater fish and fishing in Africa. Thomas Nelson and Sons (Africa) (Ptg) Ltd., Capetown, South Africa. 210 p.

KIENER, A. 1963. Famille des Salmonides. *In* Poissons pêche et pisciculture à Madagascar. Publication no. 24 du Centre Technique Forestier Tropical, 45, bis Avenue de la Belle-Gabrielle, Nogent-sur-Marne, Seine, France. 4 p. (Extract)

LEON F., J. I. 1966. Piscicultura rural en Venezuela. Bull. Office Int. Epizoot. 65(7–8): 1127–1134.

MACCRIMMON, H. R., AND T. L. MARSHALL. 1968. World distribution of brown trout, *Salmo trutta*. J. Fish. Res. Bd. Canada 25: 2527–2548.

MATSUI, Y. 1962. On the rainbow trout (*Salmo gairdnerii irideus*) in Lake Titicaca South America. Bull. Jap. Soc. Sci. Fish. 28: 497–498. (In Japanese with English summary)

MOROCCO MINISTRY OF AGRICULTURE. 1960. La pisciculture et la pêche dans les eaux continentales du Maroc. Rabat, Morocco. 67 p.

NIKOL'SKII, G. V. 1954. Special ichthyology. 538 p. (Transl. from Russian for Nat. Sci. Found., Washington, D.C., and Smithsonian Inst., by Israel Program for Sci. Transl., Jerusalem, 1961)

SERBÉTIS, C. D. 1954. La station de pisciculture du louros et la culture de la truite arc-en-ciel en Grèce, p. 17–27. *In* Conseil général des pêches pour la Méditerranée. Débats et documents techniques, No. 2, FAO (Food Arg. Organ. U.N.), Rome.

U.S. COMMISSIONER OF FISH AND FISHERIES. 1889–91. Details of distribution of food fishes 1890–91. Rep. U.S. Commissioner Fish Fish. Pt. 17: 90.

1892. Details of distribution of food fishes 1891–92. Rep. U.S. Commissioner Fish Fish. Pt. 18: lxxx.

1894. Details of distribution of food fishes, 1893–94. Rep. Commisioner Fish Fish. Pt. 20: 66, 69.

WORTH, S. G. 1893. Report on the propagation and distribution of food fishes. Rep. U.S. Commissioner Fish Fish. Pt. 19: 130, 132.

PERSONAL COMMUNICATIONS CITED

BEHNKE, R. 1969. Assistant Unit Leader, U.S. Department of the Interior, Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, Colorado Cooperative Fishery Unit, Colorado State University, Fort Collins, Colo. 80521.

BIRIBONWOHA, A. R. 1969. Principal, Fisheries Training Institute, P.O. Box 124, Entebbe, Uganda.

BOWDEN, B. J. 1969. Chairman, The Ndirande Angling Society, P.O. Box 744, Blantyre, Malawi.

CONROY, W. L. 1969. Director, Department of Agriculture, Stock and Fisheries, Konedobu, Papua.

CRICHTON, G. E. 1969. Department of Forestry and Game, Ministry of Natural Resources, P.O. Box 182, Zomba, Malawi.

DOHA, S. 1969. Reader and Head, Department of Aquiculture and Management, Faculty of Fisheries, East Pakistan Agricultural University, Mymensingh, Pakistan.

FULLER, B. R. 1969. Director of Forestry and Game, Ministry of Natural Resources, P.O. Box 182, Zomba, Malawi.

GANDARA, J. A. M. 1969. Biol., El Jefe del Departamento de Piscicultura del Instituto Nacional de Investigaciones Biologico pesqueras, Carmona Y Valle # 101 — 304, México 7, D.F.

HOYEK, B. 1969. Director of Fisheries, Ministry of Agriculture, Beirut, Lebanon.

HUSAIN, K. A. 1969. Ex-director of Fisheries, Lahore, West Pakistan.

LIVOJEVIĆ, Z. 1969. Direktor, Institut za Slatkovodno Ribarstvo, Zagreb, B. Adžije 2/V, Jugoslavije.

LUMBALA, P. 1969. République Démocratique du Congo Ministère de l'Agriculture et du Developpement Rural du Gouvernement Central, Direction des Eaux et Forêts, Kinshasa, République Démocratique du Congo.

MONTENEGRO, F. V. S. 1968, 1969. Jefe del Departamento de Piscicultura, Ministerio de Industrias Y Comercio, Quito, Ecuador.

PAZUR, K. 1969. Naučni suradnik, Institut za Slatkovodno Ribarstvo, Zagreb, B. Adžije 2/V, Jugoslavije.

RAPSON, A. M. 1969. Chief Biologist, Department of Agriculture, Stock and Fisheries, Konedobu, Papua.

ROYER, E. 1969. Fisheries Officier, Ministry of Agriculture and Fisheries, P.O. Box 470, Kingston, Jamaica.

SCOTT, D. 1969. 65, Balwearie Road, Kirkcaldy, Fife, Scotland.

SHIRAISHI, Y. 1967. Freshwater Fisheries Research Laboratory, Nikko Branch, Japan.

TERRAZAS, I. W. 1969. Bolivian Ministry of Agriculture, La Paz, Bolivia.

VELASQUEZ, F. P. 1969. Jefe Sección Pesca Continental, Instituto de Desarrollo de los Recursos Naturales Renovables, Av. Caracas No. 25A-66, Apdo. Aéreo No. 13-458, Bogotá, D.E., Colombia.

VIBERT, R. 1969. Station d'Hydrobiologie Continentale de Biarritz, Centre Scientifique — Boîte Postale No. 10, Biarritz (Basses-Pyrénées).

WOYNAROVICH, E. 1969. UNDP/TA, P.O. Box 107, Kathmandu, Népal.

An Incidence of Parasitism of Deepwater King Crab, *Lithodes aequispina*, by the Barnacle *Briarosaccus callosus*

JOHN C. MCMULLEN AND HARVEY T. YOSHIHARA

Alaska Department of Fish and Game
Kodiak, Alaska, USA

MCMULLEN, J. C., AND H. T. YOSHIHARA. 1970. An incidence of parasitism of deepwater king crab, *Lithodes aequispina*, by the barnacle *Briarosaccus callosus*. J. Fish. Res. Bd. Canada 27: 818–821.

Parasitism of commerçially important anomuran crabs in Alaskan waters by the rhizocephalan *Briarosaccus callosus* Boschma has been documented twice. One specimen from the Bering Sea was hosted by the deepwater king crab, *Lithodes aequispina*, and two others were found among a group of 99 king crab, *Paralithodes camtschatica* (Tilesius), in southeastern Alaska. In July 1969, 14 of 21 *L. aequispina* captured near Kodiak Island were found to be infected with *B. callosus*. The high incidence of infection is the first documentation of *B. callosus* in the upper Gulf of Alaska, where research on anomuran crabs has been conducted for about 12 years.

Received November 13, 1969

THREE SPECIES of commercially important anomuran crabs have been the objects of fisheries research at Kodiak Island, Alaska, for about 12 years. The king crab, *Paralithodes camtschatica*, is the dominant species, and the

Printed in Canada (J1645)

blue king crab, *P. platypus*, and the deepwater king crab, *Lithodes aequispina*, are incidentally captured.

Two incidences have been documented concerning the parasitism of Alaska king crabs by the rhizocephalan *Briarosaccus callosus* Boschma. One specimen, hosted by *L. aequispina*, was obtained in the Bering Sea (Boschma, 1962). Later, Boschma and Haynes (1969) reported two *B. callosus* from two of 99 *P. camtschatica* from southeastern Alaska.

In July 1969, 11 specimens of *L. aequispina* were captured by a tanner crab fisherman from 115 fath at 57°58′N, 153°45′W, near Kodiak Island, Alaska. During laboratory examination, 6 of the 11 specimens were found to be parasitized by adult *B. callosus*. The parasites were attached under the abdomen of each host (Fig. 1). Attachment was by means of a horny stalk,

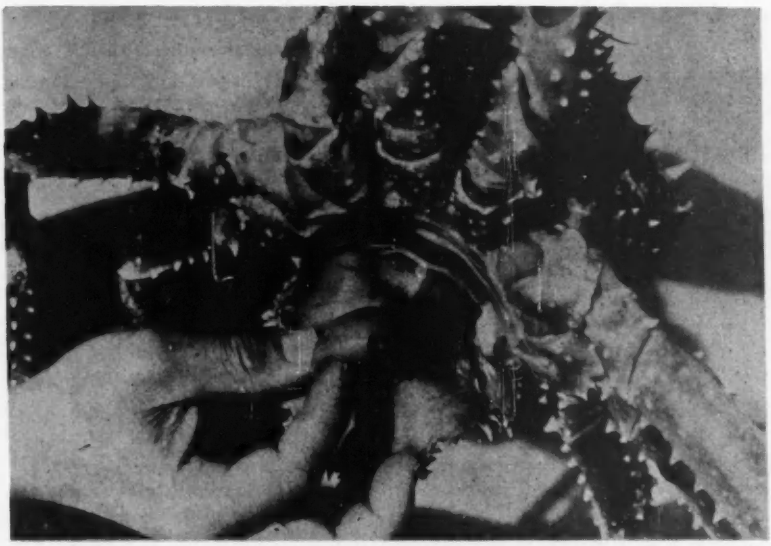


FIG. 1. Location of attachment of the rhizocephalan *Briarosaccus callosus* to the deepwater king crab *Lithodes aequispina*.

which passed into the body cavity of the host. The usual arrangement was one parasite per host, except for one crab that hosted two parasites.

Maturation of sex products was inhibited in the infected crabs, which is a known effect of parasitism by *B. callosus*. Three mature-size female hosts carried no eggs on their pleopods, nor were eggs ripening in their ovaries (Table 1). Three of four uninfected female crabs carried clutches of fertilized eggs. The fourth female probably was of immature size (108 mm carapace length), but her ovaries, like those of the three uninfected females, contained ripening ova, which would have been extruded after molting and mating in the spring of 1970. Two mature-size infected males were also dissected. Their

TABLE 1. Effects of the parasitic barnacle *Briarosaccus callosus* on sex product maturation in the deepwater king crab *Lithodes aequispina*.

Date observed (*1969*)	Male king crabs				Female king crabs			
	Specimen no.	Carapace length (*mm*) Infected	Not infected	Comments	Specimen no.	Carapace length (*mm*) Infected	Not infected	Comments
July 17	1 –	139	–	Testes not evident	1 –	116	–	Ova not ripening[a]
	2 –	136	–	Testes not evident	2 –	134	–	Egg clutch absent[b]
	3 –	116	–	–	3 –	149	–	Ova not ripening
	– 1	–	102	Immature	– 1	–	178	Egg clutch present
					– 2	–	108	Immature
					– 3	–	161	Egg clutch present
					– 4	–	142	Egg clutch present
July 22	4 –	136	–	–	4 –	131	–	Egg clutch absent
	5 –	120	–	–	5 –	115	–	Egg clutch absent
	6 –	135	–	–	6 –	135	–	Ova not ripening
	7 –	168	–	Testes not evident	7 –	123	–	Ova not ripening
	– 2	–	140	Testes very evident	– 5	–	185	Egg clutch present
Subtotals	7 males infected 2 males not infected				7 females infected 5 females not infected			

[a]Infected females without ripening ova also did not carry egg clutches.
[b]Uninfected females with egg clutches also contained ripening ova.

testes, usually very evident, were located only by careful examination. An uninfected male was not dissected, but at 102 mm carapace length was probably immature.

An Alaska Department of Fish and Game representative accompanied the fishing vessel on a second trip to obtain all specimens of *L. aequispina* that were caught. Ten mature crabs were obtained and were examined on July 22, 1969. Eight of the 10 crabs were infected by adult *B. callosus*, bringing to 14 the total of infected crabs from among 21 specimens examined. Sex product maturation in the 8 infected crabs was inhibited, as it was in the infected 6 of 11 crabs comprising the first group.

Acknowledgments — We thank Dr D. P. Henry, University of Washington, for the identification of the rhizocephalans.

REFERENCES

BOSCHMA, H. 1962. Rhizocephala. Discovery Rep. 33: 55–92.

BOSCHMA, H., AND E. HAYNES. 1969. Occurrence of the rhizocephalan *Briarosaccus callosus* Boschma in the king crab *Paralithodes camtschatica* (Tilesius) in the northeast Pacific Ocean. Crustaceana 16: 7–8.

A Generalization of the Murphy Catch Equation

PATRICK K. TOMLINSON

Operations Research Branch
Department of Fish and Game, La Jolla, California, USA

TOMLINSON, P. K. 1970. A generalization of the Murphy catch equation. J. Fish. Res. Bd. Canada 27: 821–825.

Given catches, by time intervals, from a known cohort of fish with known natural mortality rate and known fishing mortality rate for either the first or last time interval, the population size at the beginning of each interval and the fishing mortality rate during each interval may be estimated using a generalized Murphy catch equation. The generalized equation is constructed from the ratio of catches in successive intervals and depends on the assumption of simple exponential mortality within the intervals. The intervals may vary in length and have zero catches. The estimates may be badly biased depending on the nature of the catch sequence and the independent estimates of natural mortality and fishing mortality used to start the procedure.

Received December 18, 1969

MURPHY (1965, 1966) described a method of estimating mortality rates from catch data, when the catches are in numbers of fish from a known cohort. His method involves catches by time intervals, when each interval is of equal time and each contains catches. The following discussion gives a generalization of his ideas and allows for variable time intervals and zero catches. The generalized approach has been used on quarterly catches of yellowfin tuna (Davidoff,

Printed in Canada (J1678)

1969) and tag recoveries of skipjack tuna (Joseph, 1969). Also, an example problem is given to illustrate the technique.

Any period of time may be defined as having a number of units (T) where each unit is equal in time. For example, a year (period) contains 12 (T) months (unit). Furthermore, the period of time may be divided into n intervals (not necessarily of equal time) such that the ith interval contains t_i units and $\sum_{i}^{n} t_i = T$. For example, a basic unit of 1 month, $n = 4, t_1 = 3, t_2 = 3, t_3 = 6$, and $t_4 = 3$ gives a period of $T = 15$ months with 4 intervals.

To each time interval (i) belongs a catch ($C_i \geq 0$). The total catch ($\sum_{i}^{n} C_i$) must come from a single cohort of fish that is not subjected to unknown recruitment. Emigration may be treated as natural mortality if it has a constant rate and is known within each interval. Associated with each interval (i) and its catch (C_i) is a number of time units (t_i), an instantaneous fishing mortality ($F_i \geq 0$), and an instantaneous natural mortality ($M_i \geq 0$). F_i and M_i are assumed constant over the interval i.

If N_i is the number of fish in the cohort alive to begin interval i, then the number alive to begin interval $i + 1$ is given by

$$N_{i+1} = N_i \exp\left[-t_i(F_i + M_i)\right]. \tag{1}$$

The catch in interval i is given by

$$C_i = N_i E_i$$

$$E_i = F_i \left[1 - \exp\left(-t_i\left[F_i + M_i\right]\right)\right]/(F_i + M_i). \tag{2}$$

The catch in interval $i + 1$ is given by

$$C_{i+1} = N_i \exp\left[-t_i(F_i + M_i)\right] E_{i+1}. \tag{3}$$

A catch ratio (R_i) can be constructed for all but the last time interval. The ratio for interval i is given by

$$R_i = C_{i+1}/C_i = \exp\left[-t_i(F_i + M_i)\right] E_{i+1}/E_i. \tag{4}$$

If $C_{i+1} = 0$, then $E_{i+1} = 0$ and $R_i = 0$. In this latter case,

$$R_{i+1} = C_{i+2}/C_i = \exp\left[-t_i(F_i + M_i) - t_{i+1}(M_{i+1})\right] E_{i+2}/E_i. \tag{5}$$

Estimation From the Model

Of general interest is the problem of estimating the array $[F]$. If all M_i are known and either F_1 or F_n is known, all additional F_i are estimable once the R_i have been constructed from the catches.

The forward solution — If F_1 is known, the process involves estimating E_{i+1} from E_i or E_{i-1}. The equations for estimation are

$$E_{i+1} = R_i E_i \exp\left[t_i(F_i + M_i)\right];\ E_i \neq 0$$

$$E_{i+1} = R_i E_{i-1} \exp\left[t_i M_i + t_{i-1}(F_{i-1} + M_{i-1})\right];\ E_i = 0. \quad (6)$$

Once the array $[E]$ is known, the array $[F]$ may be found by iteration from

$$E_i = F_i\left(1 - \exp\left[-t_i(F_i + M_i)\right]\right)/(F_i + M_i). \quad (7)$$

The backward solution — If F_n is known, the solution is given by estimating $E_i \exp\left[t_i(F_i + M_i)\right]$ from E_{i+1} or E_{i+2}. The equations are

$$E_i \exp\left[t_i(F_i + M_i)\right] = E_{i+1}/R_i;\ E_{i+1} \neq 0$$

$$E_i \exp\left[t_i(F_i + M_i)\right] = E_{i+2} \exp\left(-t_{i+1}M_{i+1}\right)/R_{i+1};\ E_{i+1} = 0. \quad (8)$$

Once the array $E_i \exp\left[t_i(F_i + M_i)\right]$ is known, the array $[F]$ may be found by iteration from

$$E_i \exp\left[t_i(F_i + M_i)\right] = F_i\left(\exp\left[t_i(F_i + M_i)\right] - 1\right)/(F_i + M_i). \quad (9)$$

When some F_j other than F_1 or F_n is known, the array can be broken into two new arrays such that F_j is first in one and last in the other.

Possible Bias

Since Murphy's method requires all M_i and one F_i be known (in practice these are usually estimates), possible bias was investigated by writing equation 4 as

$$R_i = \frac{\exp(-F_i - \alpha - M)(F_{i+1} + \epsilon)(F_i + \alpha + M)\left[1-\exp(-F_{i+1} - \epsilon - M)\right]}{(F_{i+1} + \epsilon + M)\ (F_i + \alpha)\left[1-\exp(-F_i - \alpha - M)\right]}. \quad (10)$$

Assuming F_i is the initial known F with error α and that M is constant without error, ϵ is the error introduced in estimating F_{i+1}. Studies of equation 10 show that if $F_i \leq F_{i+1}$, then $|\epsilon| > |\alpha|$. When $F_i > F_{i+1}$, no explicit solution was found, but computer studies indicate the magnitude of the difference as well as the magnitude of the values will determine if $|\epsilon| > |\alpha|$. Similar analysis when only M is subject to error showed that all F_i are overestimated when M is too small and vice versa. Regardless of differences, α and ϵ appear to always have the same sign.

Trying to solve for a particular array of F values when F_1 is known and the trend is for increasing or steady values as time increases, it is almost im-

possible to use the forward method. Also, if a guess of F_n is made and the backward method is used, a wide range of values for F_n will result in the same estimate of F_1. Sometimes other characteristics of the data will suggest which of the F_n values is the best guess. The ideal set of data is one in which F_n is known and the values of F decrease as they go back in time. It is suggested that if this method is to be used, independent estimates of F_1 and F_n should be attempted, or some other criterion must be found to reduce the chance of bias.

Computer Program

A general computer program (FORTRAN) is available to produce the arrays $[N]$, $[F]$, and $[E]$ when the arrays $[C]$, $[M]$, $[R]$, and $[t]$ are known, along with either F_1 or F_n. The array $[R]$ may be constructed from the array $[C]$ or from other data, but the estimates of N_i depend on the C_i. The array $[E]$ is estimated from the array $[R]$ and the estimation does not depend on the array $[C]$.

Table 1. Estimates of population size (N) and and daily instantaneous fishing mortality (F) from catches (C) for 24 intervals of varying length in days (t).

Days (t)	Catch (C)	Population (N)	Mortality (F)
1	11	3206	.0035
3	146	3190	.0157
2	216	3029	.0371
1	24	2804	.0087
2	0	2775	0
1	26	2767	.0093
4	384	2737	.0380
1	17	2337	.0072
1	113	2316	.0502
3	171	2200	.0270
1	11	2019	.0053
3	144	2005	.0249
1	0	1853	0
2	39	1850	.0105
1	0	1806	0
2	67	1803	.0191
1	0	1730	0
1	61	1727	.0359
4	0	1664	0
4	113	1654	.0179
3	0	1531	0
3	76	1524	.0172
7	19	1440	.0020
4	54	1405	.0099

The program requires that $C_1 \neq 0$ and $C_n \neq 0$ (or $R_1 \neq 0$ and $R_{n-1} \neq 0$), but allows for any other $C_i = 0$ (or $R_i = 0$) as long as two consecutive zeros do not occur. The M_i and t_i may be constant or variable, but must be known. The unit of time is arbitrary, but must be consistent. The number of intervals (n) may be as great as 999. A listing of the program and instructions for its use are available from: Operations Research Branch, California Department of Fish and Game, 511 Tuna Street, Terminal Island, Calif. 90731, USA.

Example Problem

For this illustrative example, data from Parker (1955) on catches by day of marked bluegill for a 56-day period (July 9 to September 2, 1953) were grouped into 24 intervals in such a way as to have some intervals with more than 1 day and some intervals with zero catches (Table 1). Insofar as possible the intervals selected also conform to the assumption F_i is constant for the interval i. The estimate of natural mortality (0.00153) given by Parker (1955) is assumed to have been the daily instantaneous natural mortality rate and all M_i were set equal to this value. $F_1 = 0.00344$ was estimated using the catch on July 9 ($C_1 = 11$) and an estimate of $N_1 = 3210$ from Parker (1955).

The generalized Murphy method estimates of $N_1 = 3206$ and $N_{24} = 1405$ using $F_1 = 0.00344$ as a starting point, $M = 0.00153$, and forward calculations (equations 6 and 7) compare favorably with estimates ($N_1 = 3210$ and $N_{24} = 1415$) from Parker (1955). Apparently the F_i varied considerably and backward calculation is not different from forward calculation with this data. No serious bias is suspected.

REFERENCES

Davidoff, E. B. 1969. Variations in year class strength and estimates of the catchability coefficient of yellowfin tuna, *Thunnus albacares*, in the eastern Pacific Ocean. Bull. Inter-Amer. Trop. Tuna Comm. 14: 1–44.

Joseph, J. 1969. Population dynamics of the skipjack tuna (*Katsuwonus pelamis*) of the eastern Pacific Ocean. Bull. Inter-Amer. Trop. Tuna Comm. 13: 1–273.

Murphy, G. I. 1965. A solution of the catch equation. J. Fish. Res. Bd. Canada 22: 191–202.
1966. Population biology of the pacific sardine (*Sardinops caerulea*). Proc. Calif. Acad. Sci. 34: 84 p.

Parker, R. A. 1955. A method for removing the effect of recruitment on Peterson type population estimates. J. Fish. Res. Bd. Canada 12: 447–450.

Stimulation and Photography of Bioluminescence in Lanternfishes (Myctophidae)

M. ANCTIL

Department of Biology
University of Ottawa, Ottawa, Ont.

AND

C. G. GRUCHY

National Museum of Natural Sciences
National Museums of Canada, Ottawa, Ont.

ANCTIL, M., AND C. G. GRUCHY. 1970. Stimulation and photography of bioluminescence in lanternfishes (Myctophidae). J. Fish. Res. Bd. Canada 27: 826–829.

Hydrogen peroxide injections and application evoked luminescence in *Tarletonbeania crenularis taylori* and *Protomyctophum thompsoni*, but did not cause *Stenobrachius leucopsarus* to luminesce. Neither formalin nor electric shock were especially effective in stimulating luminescence in any of the above three species. The luminescent organs of myctophids were photographed by their own light.

ANCTIL, M., AND C. G. GRUCHY. 1970. Stimulation and photography of bioluminescence in lanternfishes (Myctophidae). J. Fish. Res. Bd. Canada 27: 826–829.

La luminescence de *Tarletonbeania crenularis taylori* et *Protomyctophum thompsoni* fut obtenue en injectant et arrosant les spécimens avec du peroxide d'hydrogène, tandis que la même stimulation s'est avérée inopérante sur les organes lumineux de *Stenobrachius leucopsarus*. La formaline et les chocs électriques ne produisent que peu des résultats sur la luminescence de ces trois espèces. De plus, les organes lumineux de quelques Myctophidés furent photographiés à l'aide de leur propre lumière.

Received December 10, 1969

IN THE PAST ONE HUNDRED YEARS, many scientists have studied the morphology and histology of teleost luminescent organs, but few direct observations of their bioluminescence have been published (Harvey, 1952). Although living lanternfish (Myctophidae) are not readily available, information on their luminescence has been reported (Beebe, 1934; Beebe and Vander Pyl, 1944; Nicol, 1958, 1960; Ohshima, 1911), and new data are currently being accumulated. This preliminary report is based on experimental stimulation and the photography of luminescence in three myctophid species: *Protomyctophum thompsoni* (Chapman), *Tarletonbeania crenularis taylori* Mead, and *Stenobrachius leucopsarus* Eigenmann and Eigenmann.

We carried out this work during August and September 1969, on board the C.C.G.S. *Quadra* at Ocean Station Papa in the northeastern Pacific Ocean (50°N, 145°W), about 1000 miles west of Vancouver Island. Specimens were captured with a 6-ft Isaacs-Kidd midwater trawl (by

Printed in Canada (J1670)

day) and dipnets, and neuston and plankton nets (by night). The fishes were held in refrigerated sea water (11–15 C) prior to the experiments, which were carried out in a darkroom (ambient temperatures 23–25 C). Fishes were selected from net hauls only if they appeared to be in good physical condition, and no experiments were carried out more than 2 hr after the fish had been captured. Fish captured by different techniques would be subject to differing states of "physiological shock."

Stimulation of myctophid luminescence — Harvey (1931) and Bertelsen and Grøntved (1949) reported that they induced luminescence in several stomiatoids with epinephrine injections. We tried similar experiments with myctophids, but no luminescence resulted.

We also stimulated the spinal cord of *T. crenularis taylori* and *S. leucopsarus* above the posterior end of the operculum with an electronic stimulator. Pulses of 2–20 v at frequencies of from 1 to 5/sec resulted in only brief flashes of the branchiostegal and thoracic photophores. The fish usually died within 30 sec of stimulation. This evidence gives some support to the hypothesis of nervous control of luminescence in fishes (see Nicol, 1967).

Intraperitoneal injections (0.15–0.30 cc) and external applications (0.2–0.4 cc) of hydrogen peroxide (U.S.P., 3%) evoked luminescent displays in *P. thompsoni* (3 specimens, 25–37 mm SL) and *T. crenularis taylori* (34 specimens, 34–66 mm SL), but were ineffective in *S. leucopsarus* (14 specimens, 106–108 mm SL). We found that when topical and intraperitoneal applications were done separately the latent period was longer and the resultant luminescence was less steady; therefore, after an initial experimentation period with *T. crenularis taylori*, the two were performed together.

We observed the following pattern of display in *T. crenularis taylori*. After a variable (45 sec to 3 min 30 sec) latent period, a steady but faint bluish glow appeared from a few anterior photophores, then the remaining photophores rapidly began to luminesce. At this time, a dull diffused glow marked the ventrolateral edge of the body. The glow increased in intensity, and each photophore became more sharply defined. We noted fluctuations in intensity, which seemed to be correlated with opercular movements. Usually the overall intensity decreased slightly after 3–5 min, but continuous luminescence was observed for as long as 33 min in two specimens. The caudal gland of all male fishes studied always failed to luminesce, and in specimens of either sex occasionally some lateral photophores, e.g. posterolateral, would not luminesce. On a number of occasions we placed experimental fish on their sides on a piece of white styrofoam. We noted that, when viewed laterally while they were glowing steadily, the luminescence slightly, but distinctly, illuminated the white background. This is a definite indication of a downward casting of light.

A spray of concentrated formalin evoked strong, intermittent simultaneous flashes from many photophores in *T. crenularis taylori*, and the simultaneity of response adds further support to the nervous control hypothesis. The same treatment on *S. leucopsarus* gave no response and Ohshima (1911) reported that dilute formalin had no effect on the luminescence of the so-called *Myctophum watasei* (apparently a *Diaphus*).

Crane (1968) has shown a positive response (luminescence) from hydrogen peroxide on nonglowing skin of a batfish, *Dibranchus atlanticus*, and moreover, peroxidase increased the light output of spontaneously luminescing batfish skin. The luminescent systems of *P. thompsoni* and *T. crenularis taylori* are triggered by the presence of hydrogen peroxide. There appear to be two possible mechanisms of action: either the peroxide directly stimulates the photophores, or the breakdown of the peroxide by a peroxidase system evokes luminescence. On the basis of Crane's (1968) work, the latter explanation seems more likely.

The somewhat parallel luminescent response of formalin and hydrogen peroxide on the myctophids studied suggest that not all myctophid luminescence is produced by the same biochemical system. The different pathways may be related to the evolutionary history of the Myctophidae. *Stenobrachius leucopsarus*, which did not respond to formalin and hydrogen peroxide, is generally considered to be a phylogenetically "advanced" myctophid (Fraser-Brunner, 1949) in comparison with *P. thompsoni* and *T. crenularis taylori*, which did respond to formalin and hydrogen peroxide.

Protomyctophum thompsoni and *S. leucopsarus* were captured only in the Isaacs-Kidd midwater trawl, and *T. crenularis taylori* were captured usually in a surface neuston net. This difference in capture technique might result in different physiological states of the various species, but on several occasions *T. crenularis taylori* captured in the Isaacs-Kidd midwater trawl were used for experiments and the typical results already described were apparent.

There is a possibility that some of the variation in luminescence that we observed was due to increasing dark adaptation of the investigators, eyes, but we do not feel that all variation can be attributed to this, especially as the intensity of luminescence was observed to decrease, as well as increase, during the course of each experiment.

Photography of myctophid luminescence — Luminescence in fishes has rarely been photographed. Bertelsen and Grøntved (1949) published a photograph of luminescing *Argyropelecus olfersi*, and Strum (1969) includes a photograph of the luminescence of *Porichthys notatus*. We have not been able to locate a published photograph of luminescing myctophids.

Photographs of *T. crenularis taylori* were taken using Kodak Royal-X Pan sheet film (ASA 1250) in a Busch Pressman $2\frac{1}{4} \times 3\frac{1}{4}$ sheet film camera with a Wollensak Raptar 101-mm lens. To facilitate work with living fishes the camera was prefocused to include the length of an "average lanternfish" in 2/3 of the ground glass focusing screen. A tripod supported the camera so that its plane of focus was slightly above the styrofoam sheet on which the experimental fishes were placed. Maximum lens aperture (*f*4.5) was used, and exposures were begun in total darkness after the previously described steady luminescence had begun. Exposure times were varied, but successful exposures lasted from 3 to 14 min 30 sec (Fig. 1). Typical specimen-to-lens and lens-to-film distances were each 20 cm. We found that photographs of the ventral surface (Fig. 1A) required far less exposure (3–6 min) than those of

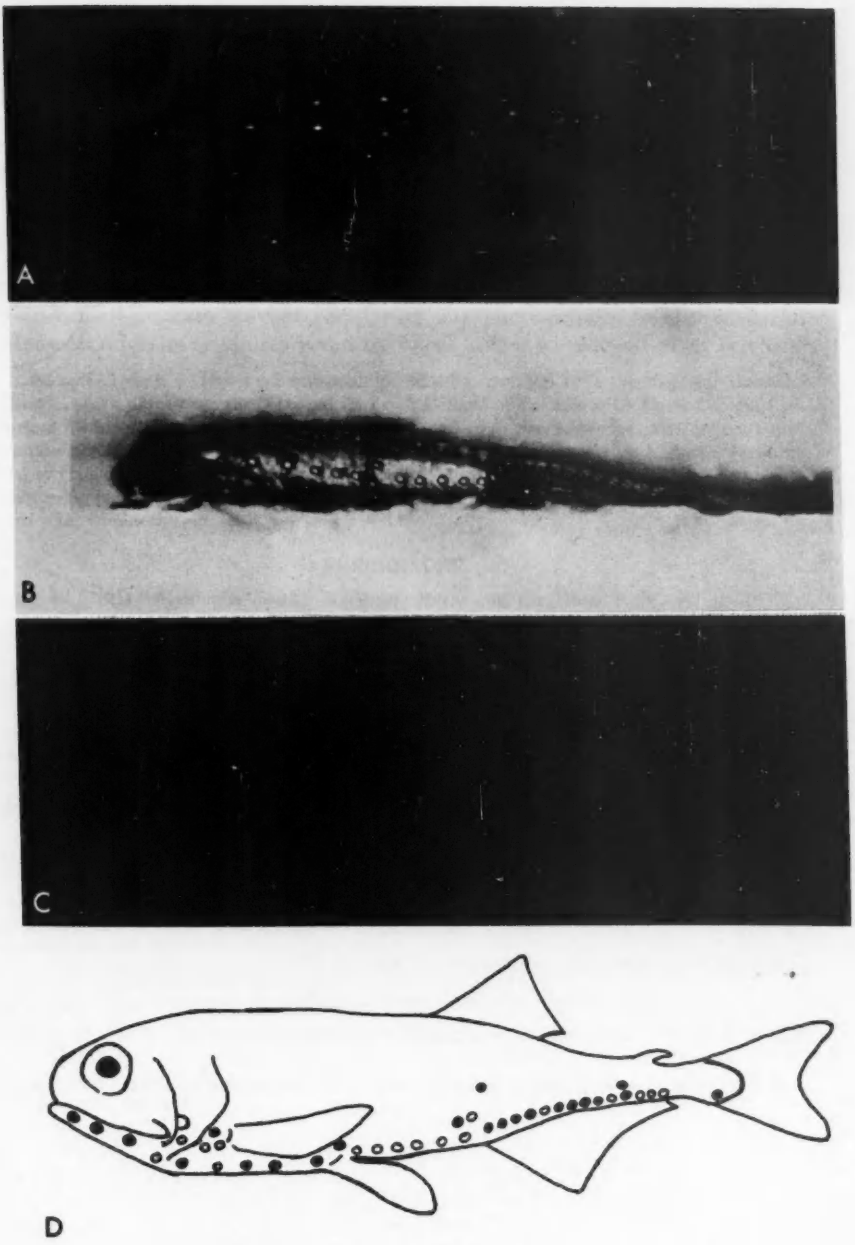


FIG. 1. (A) Ventral view of photophores of *Tarletonbeania crenularis taylori* photographed by their own light (exposure 3 min 10 sec). (B) The same specimen as in (A), but with the room lights on to show the positions of photophores on the venter of the fish. (C) Lateral view of the photophores of *T. crenularis taylori* photographed by their own light (exposure 14 min 30 sec). (D) Schematic of *T. crenularis taylori* to show position of photophores. Those photophores shown as solid dots are apparent in C, above; those shown as open circles either did not luminesce, or did not luminesce for sufficient time to register on the film.

the late
that m

Th
of the
(ASA 3
21 C) i
of Bert
of *A. a*
13.5-m
We fin
lumines

Ac
of Canad
versity o
the perso
Board of
photogra
viewed t

Beebe,

Beebe,
logic

Bertels
lecu
Kjo

Crane,
1968

Fraser-
Zoo

Harvey
logic
19

Nicol,
U.K
19
J. N
19

Ohshima
27(1

Strum,
Rec

the lateral surface (10+ min) (Fig. 1C). This supports the previous suggestion that more light is cast ventrally than laterally by the photophores.

The extreme dimness of the luminescence necessitated overdevelopment of the negatives to obtain maximum speed. Speed increases of 300–400% (ASA 3750–5000) were obtained by developing the negatives for 16 min (temp 21 C) in Edwal FG7 developer diluted 1:15 with 9% sodium sulphite. The data of Bertelsen and Grøntved (1949) show, by comparison, that the luminescence of *A. olfersi* is much brighter than that of *T. crenularis taylori*. They used a 13.5-mm lens at *f*4.5 for 22 min with a film rated at 32 Sch. for a lateral view. We find no legitimate way of comparing our photographic results with the luminescence intensity figures given by Nicol (1958) for several other teleosts.

Acknowledgments — This work was made possible by contracts from the National Museums of Canada, and a grant from the National Research Council of Canada to Dr Q. N. Laham, University of Ottawa, Ottawa, Ont. We gratefully acknowledge the assistance and cooperation of the personnel of the C.C.G.S. *Quadra* and the Pacific Oceanographic Group, Fisheries Research Board of Canada. Mr T. A. Willock criticized the manuscript and took special care to prepare photographic prints from our negatives. Drs D. E. McAllister and Q. N. Laham critically reviewed the manuscript.

REFERENCES

BEEBE, W. 1934. Half mile down. Harcourt, Brace and Co., New York. 344 p.

BEEBE, W., AND M. VANDER PYL. 1944. Eastern Pacific expeditions of the New York Zoological Society. XXXIII. Pacific Myctophidae (fishes). Zoologica 29: 59–95.

BERTELSEN, E., AND J. GRØNTVED. 1949. The light organs of a bathypelagic fish, *Argyropelecus olfersi* (Cuvier) photographed by its own light. Vidensk. Medd. Naturhist. Foren. Kjobenhavn. 3: 163–167.

CRANE, J. M., JR. 1968. Bioluminescence in the batfish *Dibranchus altanticus*. Copeia 1968: 410–411.

FRASER-BRUNNER, A. 1949. A classification of the fishes of the family Myctophidae. Proc. Zool. Soc. London 118: 1019–1106.

HARVEY, E. N. 1931. Stimulation by adrenalin of the luminescence of deep-sea fish. Zoologica 12: 67–69.
1952. Bioluminescence. Academic Press, Inc., New York. 649 p.

NICOL, J. A. C. 1958. Observations on luminescence in pelagic animals. J. Mar. Biol. Ass. U.K. 37: 705–752.
1960. Spectral composition of the light of the lanternfish (*Myctophum punctatum*). J. Mar. Biol. Ass. U.K. 39: 27–32.
1967. The luminescence of fishes. Symp. Zool. Soc. London 19: 27–55.

OHSHIMA, H. 1911. Some observations on the luminous organs of fishes. J. Coll. Sci. Tokyo 27(15): 1–25.

STRUM, J. M. 1969. Photophores of *Porichthys notatus*: ultrastructure of innervation. Anat. Rec. 164: 463–478.

Mercury Concentrations in Tissues of Fish from the Saskatchewan River

G. WOBESER,[1] N. O. NIELSEN,[1] AND R. H. DUNLOP[2]

Western College of Veterinary Medicine
University of Saskatchewan, Saskatoon, Sask.

AND

F. M. ATTON

Department of Natural Resources
Province of Saskatchewan, Saskatoon, Sask.

WOBESER, G., N. O. NIELSEN, R. H. DUNLOP, AND F. M. ATTON. 1970. Mercury concentrations in tissues of fish from the Saskatchewan River. J. Fish. Res. Bd. Canada 27: 830–834.

Muscle tissue of 81 fish from several locations in the Saskatchewan River system contained an average of more than 1.0 ppm of mercury. Fish collected at two sites had much higher concentrations, with individual fish containing up to 11.2 ppm. The concentrations found were higher than those reported for fish from uncontaminated environments, and corresponded to values reported for Scandinavian fish collected in areas of industrial pollution.

Received December 10, 1969

IN THE SCANDINAVIAN COUNTRIES, high concentrations of mercury have been found in fish from some streams and lakes (Johnels et al., 1967), in fish-eating birds in Sweden (Johnels et al., 1968), and in freshwater seals in Finland (Helminen et al., 1968). High concentrations of mercury in shellfish and fish have been reported from two areas of Japan (Kurland et al., 1960; Rucker and Amend, 1969).

There appears to be no information on the status of Canadian fish with regard to mercury concentrations in tissue. Because of the possibility of pollution of the Saskatchewan River, fish from both branches of this river were examined for the occurrence of mercury contamination.

Axial muscle was selected as the most suitable tissue for examination. Since this tissue has been used in other countries as a means of estimating the level of mercury present in the aquatic environment (Johnels et al., 1967), direct comparison would be possible.

Although tissue distribution studies suggest that muscle does not have so great an ability to concentrate mercury as some other tissues, muscle from

[1]Department of Veterinary Pathology.
[2]Department of Veterinary Physiology.

Printed in Canada (J1679)

fish in waters with moderate or no detectable contamination has a higher content of mercury than most other tissues (Johnels et al., 1967). Finally, as muscle is the tissue used for human consumption, mercury concentrations in it are of most concern.

Materials and methods — Eighty-one fish of 10 species were obtained by netting at 10 sites in the North and South Saskatchewan rivers within the province of Saskatchewan from September to November 1969. These fish were brought to the laboratory as rapidly as possible (usually within 6–12 hr) and, where necessary, were chilled with ice during transit. At necropsy, portions of liver, kidney, and muscle were placed in individual plastic bags, labelled, and frozen until analyzed. The muscle samples were taken from the longitudinal dorsal muscles on the anterior portion of the body. Scale samples were collected from all fish and used for age determination. Four fish from sources other than the Saskatchewan River were also analyzed. Two of these fish were marine fish obtained from a local retail food store. The other two fish were a rainbow trout netted in a reservoir in agricultural land near Saskatoon, and a lake trout obtained from a remote lake in northern Saskatchewan.

Livers and kidneys from 14 of the fish from the Saskatchewan River were analyzed for mercury content. These fish represented seven species and had mercury concentrations in muscle ranging from 0.5 to 10.6 ppm.

Mercury analyses were done by the method of Jacobs et al. (1960) with the following modifications. For digestion, 3 ml of concentrated sulfuric acid were used rather than 2 ml; after heating, the solution was allowed to cool for 1 hr rather than 15 min; after clearing with hydroxylamine hydrochloride solution the *p*H was adjusted to 1.0–1.5 with ammonium hydroxide. An electric furnace (type 70-T, Multiple Unit Electric Furnace, Heviduty Heating Equipment Co., Watertown, Wisconsin) was used instead of a Bunsen burner for distillation of the mercury–dithizone complex. The vapor generated in the furnace was sucked through an optical flow-through 10 × 2-cm cuvette (rather than 23 × 4-cm) in an atomic absorption spectrophotometer (Evans, EEL Model 140, Electroselenium, Ltd., Halstead, Essex, England). The light source used was a mercury element (EEL Type 2847, International Sales Associates, 115E Maple Avenue, Langhome, Pennsylvania 19047). An electronic recorder (Microcord Model 44, Photovolt Corporation, New York, N.Y.) was connected to the system to record the absorption curve. In the original method a modified mercury vapor meter was used (Jacobs et al., 1960).

In the analysis, a standard curve was obtained concurrently with each group of samples. The low standard of 0.2 μg of mercury/ml was stable and reproducible from day to day. Recovery of mercury added to animal tissues in the form of mercury chloride ranged from 88 to 99%.

Samples of muscle from six of the fish from the Saskatchewan River were analyzed at the Analytical Laboratory, Department of Chemistry and Chemical Engineering, University of Saskatchewan, Saskatoon, Sask., without knowledge of the results obtained in our laboratory. The method employed in that laboratory involved hot digestion of the sample with nitric acid, oxidation with perchloric acid, extraction with dithizone, flash decomposition of the mercury–dithizone complex by heating to produce mercury vapour, and estimation of mercury by atomic absorption spectrophotometry.

The concentrations of mercury (ppm) in the six fish as determined by the two laboratories were:

Fish no.	A	B	C	D	E	F
Veterinary Physiology	2.1	0.9	5.5	10.5	5.3	11.2
Chemistry and Chemical Engineering	2.2	0.5	5.7	8.0	6.5	6.8

Rucker and Amend (1969) observed that most of the mercury in muscle is in the blood in the muscle. If this is correct, the variation in results of the two laboratories may have been due to difference in content of blood of various portions of muscle.

All mercury concentrations, unless otherwise stated, are on a wet-weight basis. Common names of fish species are those used by the American Fisheries Society (Bailey et al., 1960).

Results — The concentration of mercury in muscle tissue of fish from the Saskatchewan River appeared to be high (Table 1); and varied greatly among the fish from different sites. There were wide variations in mercury content of fish of the same species from the same site, but there appeared to be little

TABLE 1. Mercury concentrations (averages and, in parentheses, ranges) in muscle from various species of fish taken from 10 sites on the Saskatchewan River during 1969.

Species	No. fish	Mercury (*ppm*)
North Saskatchewan River		
Alberta – Saskatchewan Border (Oct. 19)		
Goldeye	2	1.0(0.9–1.1)
Northern pike	2	1.2(0.7–1.6)
Sauger	2	1.3(1.1–1.5)
Walleye	2	1.0(0.6–1.4)
White sucker	2	0.0(0.7–1.1)
Avg		1.1
North Battleford (Oct. 19)		
Goldeye	1	1.0
Longnose sucker	1	0.7
Northern redhorse	1	2.4
Walleye	3	0.9(0.6–1.3)
White sucker	1	1.1
Avg		1.1
4 miles above Prince Albert (Oct. 20)		
Goldeye	2	2.5(2.2–2.8)
Northern pike	2	1.1(1.0–1.1)
Avg		1.8
Cecil Ferry (Sept. 24)		
Goldeye	4	2.0(1.1–2.4)
Longnose sucker	4	1.0(0.8–1.3)
Sauger	1	1.4
Walleye	2	2.1(1.4–2.8)
White sucker	4	4.3(0.9–5.5)
Avg		1.6
South Saskatchewan River		
Leader Ferry (Oct. 29)		
Goldeye	3	1.5(1.3–1.6)
Longnose sucker	2	1.3(0.8–1.7)
Mooneye	2	2.6(1.6–3.6)
Northern pike	1	1.0
Northern redhorse	2	1.0(1.0)
Avg		1.5
1 mile above Saskatoon (Oct. 5–Nov. 11)		
Northern pike	4	1.0(0.5–1.7)
Clarkboro Ferry (Oct. 7)		
Burbot	1	5.3
Goldeye	2	4.2(1.2–7.2)
Northern pike	3	9.1(6.1–10.6)
Walleye	2	4.9(4.4–5.3)
White sucker	2	8.3(5.3–11.2)
Avg		6.7
Saskatchewan River		
Cumberland House (Nov. 3)		
Goldeye	11	5.0(2.1–11.0)
Tobin Lake (Nov. 7)		
Burbot	1	1.2
Goldeye	3	1.3(0.9–1.9)
Northern redhorse	1	2.1
Walleye	2	2.7(2.6–2.7)
Yellow perch	1	1.4
Avg		1.8

difference between species. There appeared to be no association between mercury concentration and age.

The mercury concentrations found in muscle, livers, and kidneys of 14 fish from the Saskatchewan River were

Muscle	10.5	10.6	6.1	4.4	5.3	1.2	5.3	11.2	5.3	1.1	1.5	0.6	1.0	0.5
Liver	14.3	12.8	13.4	10.4	12.7	1.2	4.9	16.5	8.7	2.5	1.9	2.5	1.6	1.3
Kidney	13.1	21.1	20.5	10.5	9.5	1.2	–	13.4	6.0	1.6	2.3	1.4	1.4	–

The four fish from sources other than the Saskatchewan River were also found to contain mercury. The concentrations (ppm) in muscle of these fish were halibut (obtained from retail store), 0.3; bluefish (obtained from retail store), 0.6; rainbow trout, 1.0; and lake trout, 0.8.

Discussion — The results of this preliminary study indicate that abnormal concentrations of mercury occur in fish in at least part of the Saskatchewan River system with very high levels occurring in some areas. The concentrations of mercury found in all fish analyzed were higher than those reported for normal freshwater fish (0.05 ppm, Rucker, 1968; 0.2 ppm, northern pike in Sweden, Johnels et al., 1968; 0.2 ppm, rainbow trout, Rucker and Amend, 1969; 0.09 ppm, Sweden, Johnels et al., 1967). The concentrations corresponded to those found in Sweden in fish from waters directly contaminated by industrial effluents (1.21 ppm, Johnels et al., 1967; up to 8 ppm, Berglund and Wretling, 1967) but were lower than those reported for shellfish and fish from Minimata Bay, Japan (10–40 ppm wet-weight basis, Rucker, 1968; 33–150 ppm dry-weight basis, Saito et al., 1961; 27–102 ppm wet-weight basis, Kurland et al., 1960).

Johnels et al. (1967) found significant concentrations of mercury (0.75–1.1 ppm) in fish from apparently uncontaminated waters in Sweden. They speculated that these concentrations could be the result of the influence of mercury in the bedrock, or, alternatively, aerial fallout from unidentified industrial sources. The latter was considered the more likely explanation. No information appears to be available on the mercury content of Saskatchewan soils or bedrock. The bedrock is primarily sedimentary (Richards and Fung, 1969, p. 236), which in general has a higher mercury content than does igneous rock (James, 1962).

The wide variation in mercury concentration within species from the same site agrees with the observations of Hannerz (1968) who found as much as a 10-fold variation in mercury content among fish of the same species given identical exposure to mercury. This author also found that direct accumulation from the environment through the epithelia was the important route of accumulation, which may explain the lack of specific variation, even among species with very different food habits.

The mercury content of muscle tissue of fish from contaminated environments has been reported to increase with age (Johnels et al., 1967). No such association was observed in the present study. However, the numbers of fish of any one species from a single location were small and the age distribution was influenced by the type of net used for collection.

Livers and kidneys of the 14 fish from which these tissues were analyzed contained an average of 1.9 and 1.7 times as much mercury respectively as did muscle from the same fish. Similar values have been reported by Hannerz (1968).

At the present time, Canada does not have a maximum allowable concentration of mercury in food, although tolerances have been set for various other metals (Department of National Health and Welfare of Canada, 1969).

Berglund and Wretling (1967) stated that fish with a mercury content of 1.0 ppm or higher (assuming daily consumption) should be considered unusable for human food. If this value is correct, many fish from the Saskatchewan River are unsuitable for human food.

Acknowledgments — This work was supported by grants from the Medical Research Council of Canada (Grant no. 2002) and from the Institute for Northern Studies, University of Saskatchewan.

We express our thanks to Miss J. E. Massey, Department of Veterinary Physiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, for analysis for mercury of the specimens reported here, and the staff of the Fisheries Branch, Department of Natural Resources, Province of Saskatchewan, for collection of specimens and age determination of the fish.

REFERENCES

BAILEY, R. M., E. A. LACHNER, C. C. LINDSEY, C. R. ROBINS, P. M. ROEDEL, W. B. SCOTT, AND L. P. WOODS. [ed.] 1960. A list of common and scientific names of fishes from the United States and Canada. 2nd ed. Amer. Fish. Soc. Spec. Publ. 2: 102 p.

BERGLUND, F., AND A. WRETLING. 1967. Toxikologisk vardering au kvicksilver-halter i svensk fisk. Var Foda 19: 9.

DEPARTMENT OF NATIONAL HEALTH AND WELFARE OF CANADA. 1969. Office consolidation of the Food and Drugs Act and of the Food and Drug Regulations, with amendments to March 11, 1969. The Queen's Printer, Ottawa. 184 p.

HANNERZ, L. 1968. Experimental investigations on the accumulation of mercury in water organisms. Rep. Inst. Freshwater Res. Drottningholm 48: 120–175.

HELMINEN, M., E. KARPPANEN, AND J. I. KOIVISTO. 1968. Saimaan norpan Elohopeopitoisuudesta, 1967. Suomen Elainlaakarilehti Finsk Veterinartidskr. 120: 87–89.

JACOBS, M. B., S. YAMAGUCHI, L. J. GOLDWATER, AND H. GILBERT. 1960. Determination of mercury in blood. Ind. Hyg. Ass. J. 21: 475–480.

JAMES, C. H. 1962. A review of the geochemistry of mercury and its application to geochemical prospecting. Imp. Coll. Sci. Technol. London Tech. Commun. 41: 42 p.

JOHNELS, A. G., M. OLSSON, AND T. WESTERMARK. 1968. *Esox lucius* and some other organisms as indicators of mercury contamination in Swedish lakes and rivers. Bull. Office Int. Epizoot. 69: 1439–1452.

JOHNELS, A. G., T. WESTERMARK, W. BERG, P. I. PERSSON, AND B. SJOSTRAND. 1967. Pike (*Esox lucius* L.) and some aquatic organisms in Sweden as indicators of mercury contamination in the environment. Oikos 18: 323–333.

KURLAND, L. T., S. N. FARO, AND H. SIEDLER. 1960. Minimata disease. The outbreak of a neurologic disorder in Minimata Japan, and its relationship to the ingestion of seafood contaminated by mercuric compounds. World Neurol. 1: 370–395.

RICHARDS, J. H., AND K. I. FUNG. 1969. Atlas of Saskatchewan. Modern Press, Saskatoon, Sask.

RUCKER, R. R. 1968. Effects of mercurial compounds on fish and humans. Bull. Office Int. Epizoot. 69: 1431–1437.

RUCKER, R. R., AND D. F. AMEND. 1969. Absorption and retention of organic mercurials by rainbow trout and chinook and sockeye salmon. Progressive Fish-Cult. 31: 197–201.

SAITO, M., T. OSONA, J. WATNABE, T. YAMAMOTO, M. TAKEUCHI, Y. OHYAGI, AND H. KATSUMMUMA. 1961. Studies on Minimata disease. I. Establishment of the criterion for etiological research in mice. Jap. J. Exp. Med. 31: 277–290.

Recent Articles on FRB Investigations Published in Other Periodicals

Until 1970, reprints of these articles have been included in an annual volume of "Studies," composed of two parts. The final binding is the 1969 Studies, Part 2, which included No. 1355. These will no longer be bound and distributed. We will continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating FRB establishment. The boldface number following each title refers to the FRB establishment listed below.

(1) Fisheries Research Board of Canada
Biological Station
P.O. Drawer 100
Nanaimo, British Columbia

(2) Fisheries Research Board of Canada
Vancouver Laboratory
6640 N.W. Marine Drive
Vancouver 8, British Columbia

(3) Fisheries Research Board of Canada
Freshwater Institute
501 University Crescent
Winnipeg 19, Manitoba

(4) Fisheries Research Board of Canada
Arctic Biological Station
P.O. Box 400
Ste. Anne de Bellevue, Quebec

(5) Fisheries Research Board of Canada
Biological Station
St. Andrews, New Brunswick

(6) Fisheries Research Board of Canada
Marine Ecology Laboratory
Bedford Institute
P.O. Box 1006
Dartmouth, Nova Scotia

(7) Fisheries Research Board of Canada
Halifax Laboratory
P.O. Box 429
Halifax, Nova Scotia

(8) Fisheries Research Board of Canada
Biological Station
St. John's, Newfoundland

Studies No.

1408. TARR, H. L. A. 1967. Purine nucleoside phosphorylases, p. 113–118. *In* L. Grossman and W. Moldave [ed.] Methods in enzymology, Vol. XII. Academic Press, Inc., New York, N.Y. (2)

Guide to Authors

Details on typing and other items in preparing copy are given in a guide for typists. Also, a list of recommended spellings of about 100 words common in fisheries journals is available from the Office of the Editor.

EDITORIAL PRACTICES

An abstract must be provided in the same language as the text; authors are encouraged to provide both English and French versions.

The *Style Manual for Biological Journals* (2nd ed. American Institute of Biological Sciences, 2000 P St. N.W., Washington, D.C.) is, with few exceptions, used as the authority in matters of form. *Webster's Third New International Dictionary* is the standard for spelling and division of words in English; *le Grand Larousse Encyclopedique*, for those in French. Exceptions are made for manuscripts that follow the *Oxford English Dictionary* entirely. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. Manuscript reports are included in the list of references, but are identified both there and in the text by the letters "MS" before the year.

Notes are distinguished from *papers* on the basis of scope or completeness, rather than quality, of the research. They may be on problems with narrow, readily defined limits; on fishing gear or methods, or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued before completion; or on preliminary findings that warrant publication before problems are resolved satisfactorily. Or they may be used to establish priority in publication. They may be organized with as much flexibility as papers except that they may have no centered headings. They should take no more than four pages in the *Journal*.

In general, *series titles* should be avoided.

Illustrations

Line drawings are preferably made with black india ink on white drawing or tracing paper or on coordinate paper with blue grids. All lines, symbols, letters, and numerals must be neat, and legible when the illustration is reduced to the appropriate size. Drawings should be grouped, when feasible, for economy of space. Originals must be submitted and should be less than 20 inches in the greater dimension for full-page illustrations, and proportionately smaller for those taking less than a page. Drawings must not be rolled for mailing.

Prints of photographs should be made on glossy paper with a wide range in tone. They should be cropped, squared, and mounted with rubber cement on white cardboard, with no space between those arranged in groups.

Proofs and Reprints

Galley proof is sent to the author, and he is responsible for detecting errors in printing as well as in content.

Fifty reprints, without covers, are supplied free. Additional copies needed must be ordered, on the form provided, when the galley proof is returned.

CHECKLIST ON MANUSCRIPTS

Attention to the following questions will expedite appraisal of manuscripts by referees and editors. The same questions are recommended to referees who review manuscripts for the *Journal*.

General

Is there new knowledge in the report worth publishing?

Are the findings, interpretations, and conclusions sound, and relevant to the purpose of the study?

If the manuscript is merely confirmatory, is it worth publishing?

Does the manuscript need to be rewritten before the work can be assessed?

Is the contribution placed in the proper perspective concerning the state of knowledge of the subject?

Is the manuscript too long?

Are all the tables and figures needed, and organized for economy of space?

Would any of the text be clearer if condensed?

Should some of the data be made available separately, in a manuscript report or at a data repository?

Should the manuscript be condensed as a Note?

Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Are summary statements given at the beginnings of sections and paragraphs, and are details in sections and paragraphs relevant to their topics?

Are there inconsistencies between tables or figures and the text, or within the text?

If the manuscript is from a thesis, does it retain characteristics common in these but not desirable in journals?

Parts of the Manuscript

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Has the *introduction* all the important guideposts for orienting the reader?

Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data obtained?

Are generalizations on the *results* adequate?

Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

If there are loopholes in interpretation, are they acknowledged? Should they be overcome?

Should other interpretations be recognized?

Guide for Typists Preparing Copy for

Journal of the Fisheries Research Board of Canada

Read the "Guide to Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 8.5 × 11 inches (21.5 × 28 cm). If possible, use paper with the lines numbered in the margin. Leave all margins at least 1 inch (2.5 cm) wide. Use a nylon or carbon ribbon and keep the typewriter keys clean.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word and proper names and adjectives. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numbers. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (Appendix A). Type the abstract on a separate page (Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send two copies (the ribbon copy and a carbon copy or a photocopy) to the Office of the Editor.

Tables Examine the current volume of the *Journal* to see how tables are set up (Appendix C). Note especially the following concerning *numbered tables*:

Type each table on a separate page, using paper without the lines numbered.

Number the tables with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, they are typed with the text, they seldom need horizontal lines, and the data are arranged in as few rows as possible (Appendix C).

Illustrations Send the original and two clear copies of each illustration with the manuscript. At the lower left of each figure and copy, give the author's name and the figure number. Also type each caption with its figure for identification. For photographs, type this information on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for Illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical expressions, equations, and formulas Leave three spaces above and below lines that have much material in superscripts or subscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter l and the numeral one, and between the capital letter O and the numeral zero. Make other explanatory notes similarly.

References Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style that is used in the *Journal* (Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list, but if an abbreviation is in doubt, type the name in full.

Spellings A list of about 100 words common in fisheries journals is given in Appendix F.

Appendices Appendices A to F are available on request from the Office of the Editor.

New!
Fisheries Research Board of Canada Bulletin
Now Available!

The Harbour Seal in British Columbia

Michael A. Bigg

No. 172 33 pages $2.00

The results of a field study made on the harbour seal, Phoca vitulina richardi, in British Columbia are reported and discussed. The study, based on field observations and a collection of 415 specimens, describes the method of determining age, the reproductive cycle and ages at sexual maturity, prenatal and postnatal growth, and population composition and dynamics.

Available from the Queen's Printer for Canada, Ottawa, and from our sales agents, or the following Queen's Printer for Canada bookshops:

— Halifax: 1735 Barrington St.
— Montreal: Courses Vie Bldg., 1182 St. Catherine St. West
— Ottawa: Daly Bldg., corner Mackenzie and Rideau
— Toronto: 221 Yonge St.
— Vancouver: 657 Granville St.
— Winnipeg: 393 Portage Ave. (at Edmonton)